

ACTA PATHOLOGICA ET MICROBIOLOGICA SCANDINAVICA

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VOL. XXI

FASC. 2. 1944.

EINAR MUNKSGAARD · KØBENHAVN

MCMXLIV

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ACTA PATHOLOGICA ET MICROBIOLOGICA SCANDINAVICA

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In ACTA PATHOLOGICA ET MICROBIOLOGICA SCANDINAVICA only articles written by Scandinavian authors are published; they are issued in English, French or German, according to the author's desire.

Subscribers are requested to apply to *Ejnar Munksgaard*, Publisher, Copenhagen, Nørregade 6. One volume (generally 4 numbers, ca. 6—700 pages) is published every year with numerous supplements. Each volume costs 35 Danish crowns.

Dans ACTA PATHOLOGICA ET MICROBIOLOGICA SCANDINAVICA ne sont publiés que des articles écrits par auteurs scandinaves; selon leur désir, ils seront publiés en français, anglais ou allemand.

Pour les abonnements on est prié de s'adresser au éditeur, *M. Ejnar Munksgaard*, Copenhague, Nørregade 6. Prix par volume Cr. Dan. 35.—

In ACTA PATHOLOGICA ET MICROBIOLOGICA SCANDINAVICA werden nur Artikel von skandinavischen Verfassern veröffentlicht; den Wünschen der Verfasser gemäss erscheinen sie in deutscher, englischer oder französischer Sprache. Zu beziehen von der Verlagsbuchhandlung *Ejnar Munksgaard*, Kopenhagen, Nørregade 6. Preis pro Band 35 dänische Kronen.

1 ACTA PATHOLOGICA ET MICROBIOLOGICA SCANDINAVICA optages Afhandlinger større end 2 Ark (32 Sider). Manuskripter overgives til Engelsk, Fransk eller Tyisk, til en af

A STANDARD FOR ANTISTREPTOLYSIN O OF HUMAN SERUM, AND ITS PRACTICAL APPLICATION

By *Johs. Ipsen.*

(Received for publication May 29th, 1943).

Among the toxins formed by hemolytic streptococci the oxygen-labile hemolysin, the O-streptolysin found by *Todd*, has in recent years achieved practical clinical significance. Its pronounced antigenic character causes the formation of a specific antibody the occurrence of which in serum from patients attracts clinical interest inasmuch as an increase of the antistreptolysin O in the serum beyond a certain limit is a strong indication of an existing infection with hemolytic streptococci (*Coburn and Pauli, Gordon and Balteanu, Winblad, Kalbak, and others*).

As a measure of the amount of antistreptolysin *Todd* defined a unit along the same principles as those which apply to the unit designation of internationally standardized antitoxins, rating the potency of a strong antitoxin-containing rabbit serum at 20,000 units per cubic centimeter. By means of this serum it is possible to determine the lysin dose which is neutralized by 1 unit and with this dose to assay and express the potency of other sera in units per 1 cc (the antistreptolysin titer).

At the State Serum Institute — in the diagnostic department of which 50 to 100 antistreptolysin titrations are made every day — *Todd's* unit is used in specifying the titer, Dr. *Kalbak* having obtained a small portion of *Todd's* original rabbit standard in 1939. To insure stable storage of this serum it was diluted 1:20 and distributed on 1 cc ampoules in which it was desiccated to constant weight.

Since the quantity of this standard serum was very limited and could not be supplemented, the question arose of preparing, before it was too late, another standard serum which could be accurately standardised against the original. This task was referred to the department of biological standards which already kept the international-

ly adopted standards for other antitoxins. It would seem natural to choose a serum preparation from rabbits immunized with 0-streptolysin, just as *Todd's* standard is prepared, but after Dr. *Kalbak*, induced by the author, had demonstrated that the antistreptolysin in human serum and rabbit serum had qualitatively different reaction curves it became obvious that a standard of human serum would be preferable. The difference in the reaction curves causes the fluctuations in the sensitivity of the blood corpuscles to influence the result of a comparison between rabbit serum and human serum, and since the standard serum first of all is to serve in the assay of human sera it is natural to choose a human serum as standard.

We know several examples which show that biologically active substance of the same kind may exhibit a qualitative difference when their origin is different. Thus *Bjorneboe* has demonstrated the existence of a difference in the slope of the reaction curves for the pneumococci antibody (protection experiments on mice) from rabbit serum and horse serum. The diphtheria and the tetanus antitoxins have different reaction curves when they are obtained from guinea pigs and horses, a difference which is especially pronounced in the beginning of the immunization (*Ipsen* (1942)). It may be mentioned, moreover, that the slope of the reaction curve for the thyrotropic hormone differs all according to whether the preparation is obtained from pig-pituitary or ox-pituitary glands (*Ib Andersen*).

Preparation of the standard. Three patients with strongly increased antistreptolysin titer were bled by Dr. *Kalbak*, a couple of hundreds cc being obtained from each. The serum in question was mixed and diluted with physiological sodium chloride solution (330 cc of serum made up to 1 liter). The diluted solution was Seitzfiltered and distributed on 5 cc ampoules. These ampoules were transferred to desiccator and dried to constant weight. 5 of them were removed, their contents weighed and dissolved in 5 cc of saline solution, whereupon the antistreptolysin content of these solutions was compared with *Todd's* rabbit standard.

The reaction curve for the antistreptolysin. In order to establish an exact measure of the above mentioned difference between the quality of the two sera it was necessary to study the reaction curve by means of a somewhat detailed method which aims at theoretical conditions, but is unnecessary in the practical routine assay of sera for the purpose of diagnosis.

The comparison of the two standards is based on the usual assaying principle: Diminishing doses of serum are added to a constant dose of the toxin, and saline solution is added to constant volume in all glasses. After a certain time of combination rabbit's blood is added to the toxin-antitoxin mixtures, and, if the serum doses are suitable, all degrees from no hemolysis to complete hemolysis will be found in the series. By centrifuging the non-hemolysed blood corpuscles down and comparing the colour of the supernatant fluid

with that of a completely hemolysed sample in a colorimeter it is possible to express the degree of hemolysis in each glass as percentage of the total hemolysis.

If these degrees of hemolysis are plotted against log serum dose the same shape of curve will be found for sera of the same quality, independently of the potency of the serum, it being possible by parallel displacement to superpose the curves for two sera of the same quality. It is found, however, that rabbit serum and human serum do not have congruous reaction curves. For a closer study of these curves we employ another expression for the reaction, an expression having a more constant relation to the serum dose than the degree of hemolysis.

The magnitude of the degree of hemolysis is dependent on how much toxin is not combined with the antitoxin after the reaction of the mixture is complete. But the amount of »free« toxin has widely different effects on different blood samples, varying from animal to animal and from day to day with respect to mean resistance and standard deviation of resistance. The reaction curve will therefore be different as to slope and position for each new blood sample investigated, despite the fact that the same amount of toxin must be assumed to be free in the same mixture of toxin and antitoxin.

It is therefore expedient in each experiment to test the resistance of the blood sample against a pure toxin series, adding the same amount of blood to diminishing doses of toxin in the same volume as in the antitoxin experiment. A curve representing the relation between dose of toxin and degree of hemolysis may then be recorded each time, and from this curve we may read the amount of toxin that corresponds to a certain degree of hemolysis.

Besides by this purely graphical method it is also possible to obtain a more exact expression arithmetically by converting the degree of hemolysis into probits and plotting these probits against log dose of toxin. The result is a linear reaction curve which facilitates the interpolation (for details, see *Ipsen* (1941)).

The amount of free toxin may thus be substituted in the reaction curve for the antitoxin instead of the degree of hemolysis, and a curve obtained which is independent of biological variations and which expresses the physico-chemical combination between toxin and antitoxin. This curve must be the same each day, and it is therefore possible from the collected experiments to build up a curve for rabbit and human antistreptolysin.

Fig. 1 shows two experiments on different days where the blood corpuscles exhibited a wide difference in sensitivity to the streptolysin, as seen from the curves on the right. By lysin dose 1.0 we understand the dose which is used in the antitoxin experiment (test dose). On April 28th lysin dose 0.22 produced a degree of hemolysis

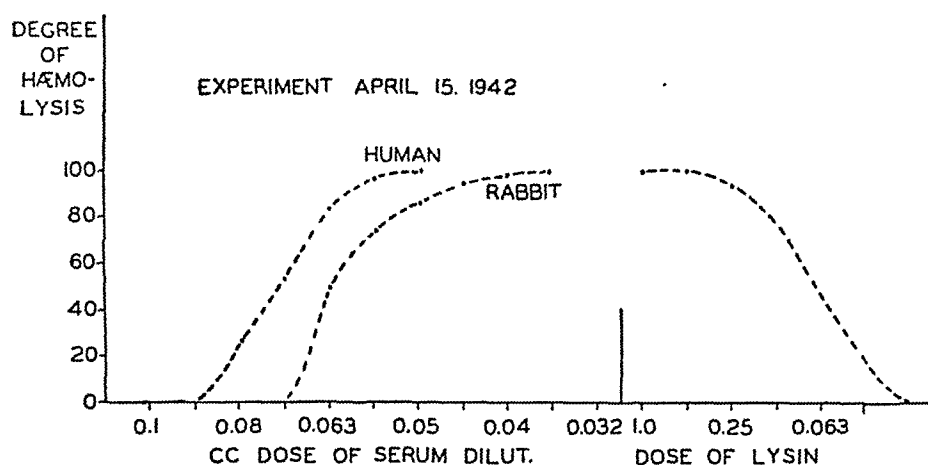
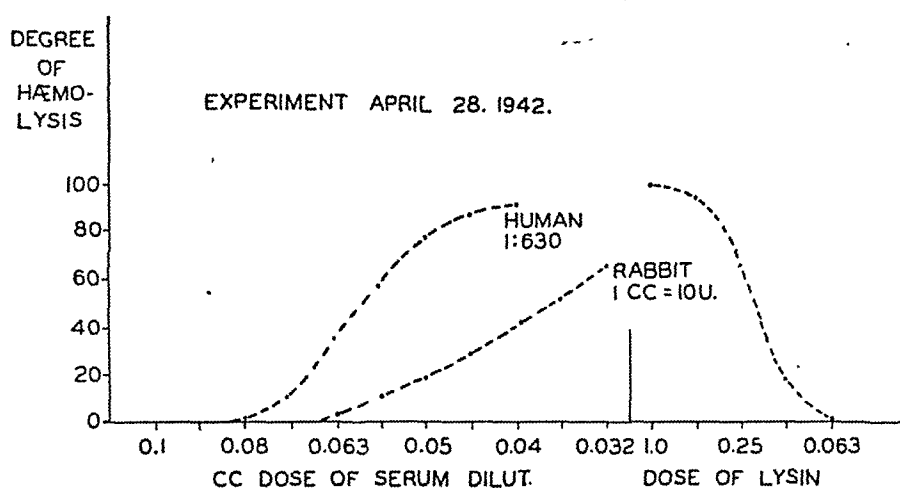


Fig. 1.

Reaction curves for human and rabbit standard. Degree of hemolysis plotted against log dose of serum, resp. log dose of lysin.

amounting to 50 % while the blood corpuscles on April 15th were so sensitive that 0.07 test dose was enough to produce 50 % hemolysis. For this reason the curves for the two antitoxins are widely different on the two days, but if the hemolysis percentages are replaced by the amount of free toxin, which can be read on the curves at the right, the same curve shape is obtained for each of the two sera on both days of experiment. This is shown in fig. 2 where both experiments are entered on the same curve.

It was now found in numerous experiments that it was possible each day to reproduce characteristic curves for the relation between the amount of free toxin and the doses of the two sera, the shape being the same as that of the curves in fig. 2. Both of these curves

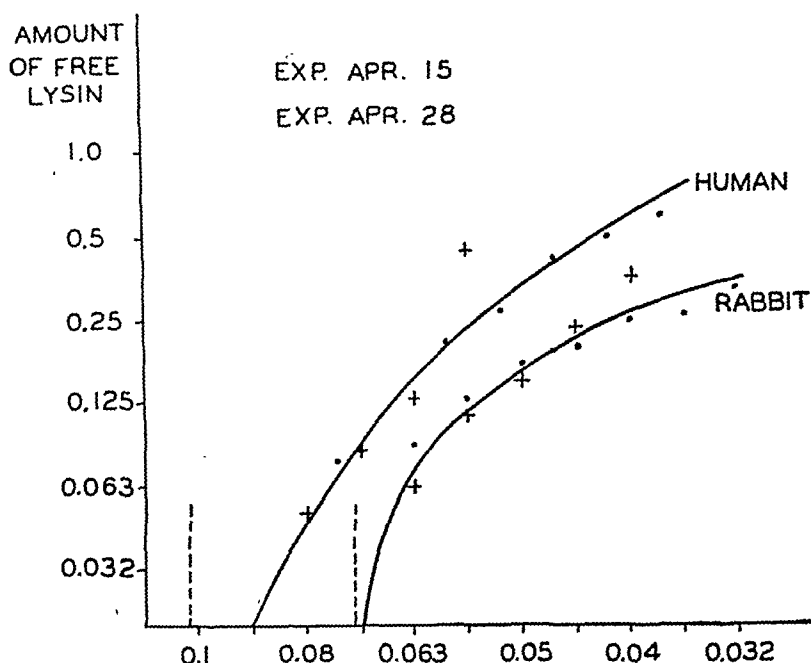


Fig. II.

Reaction curves for human and rabbit serum. Log dose of free toxin plotted against log dose of serum. The curves of the two experiments are displaced along the axis of abscissae.

show a tendency to approach two asymptotes, one parallel to the axis of ordinates and one to the axis of abscissae. The vertical asymptote intersects the axis of abscissae at the serum dose which just neutralizes the total amount of toxin added, while the horizontal asymptote necessarily corresponds to the total amount of toxin used. Between these two asymptotes both curves show a positive curvature, but to a different degree.

The ratio between the potencies of the two sera will therefore depend upon the degree of reaction — amount of free toxin — chosen for the comparison. The ratio assumes a minimum value if the amount 0.10 is chosen, the distance between the curves being minimum at this ordinate. This degree of reaction is purely arbitrary, however; it is of greater interest to compare the serum doses that just show complete neutralization, which graphically means to determine the distance on the curve between the vertical asymptotes of the two sera.

This comparison between the two sera was made several times after the curves for the two sera had been drawn once for all. Each experiment was recorded as a curve showing the relation between free toxin and serum, the scale being the same as for the standard curve. These curves were traced on transparent paper and placed on top of the corresponding standard curve; having arrived at the best

Table I.
Comparative Assay of Human and Rabbit Standard Antistreptolysin.

Date 1942	Lysin and dose of lysin		Ampoule no.	Neutralising dose of		Dilution of human serum	Potency of human se- rum Units/c. c.	Weight of dry substance	Mgms. of dry substance
				human serum	rabbit Serum				
17/3	No. 39	0.10 cc	5	0.089 cc	0.73 Units	1:63	517	37.26 mgrm	0.072
19/3	—	—	5	0.117 —	0.73 —	1:63	394	37.26 —	0.095
26/3	—	—	2	0.099 —	0.73 —	1:63	465	37.10 —	0.080
30/3	—	—	3	0.099 —	0.73 —	1:63	465	37.44 —	0.081
31/3	—	—	4	0.092 —	0.73 —	1:63	500	37.34 —	0.075
1/4	—	—	4	0.102 —	0.73 —	1:63	452	37.34 —	0.083
15/4	—	—	2	0.096 —	0.73 —	1:63	479	37.10 —	0.078
17/4	—	—	2	0.095 —	0.73 —	1:63	484	37.10 —	0.077
27/4	—	—	5	0.096 —	0.73 —	1:63	479	37.26 —	0.078
28/4	—	—	2	0.103 —	0.73 —	1:63	447	37.10 —	0.083
24/7	No. 49	0.13 cc	2	0.124 —	0.80 —	1:63	407	37.10 —	0.091
25/7	—	—	2	0.127 —	0.80 —	1:63	397	37.10 —	0.093
21/7	—	0.10 cc	2	0.069 —	0.47 —	1:63	429	37.10 —	0.087
31/7	—	0.33 cc	2	0.197 —	2.14 —	1:45	489	37.10 —	0.076
11/9	—	0.28 cc	4	0.227 —	2.29 —	1:50	505	37.34 —	0.074
17/9	—	—	4	0.237 —	2.29 —	1:50	483	37.34 —	0.077

Geometric mean: 0.0809
 Logarithmic standard dev.: 0.038
 (9.2%)
 Log. error of mean:
 0.0095
 (2.2%)

1 unit = 0.0809 mgrm \pm 0.0018

fitting to the results the serum dose corresponding to the vertical asymptote could be read. The two serum doses found each day are recorded in table I, and the potency of the human serum calculated in units of the rabbit serum. The experiment is carried out with 4 different ampoules of the desiccated human serum. From the potency of the dissolved the potency of the dry substance is then calculated in units per milligram. Different lysin preparations were used and the test dose was varied in some of the experiments.

The 16 determinations of the weight of dry preparation that corresponds to 1 unit, and which are reported in the last column, vary within the limit permitted by the experimental error. The standard deviation of the logarithm of these values (0.038) corresponds exactly to the standard deviation given by *Kalbak* (0.0375) and calculated on the basis of repeated assays of several human sera against the rabbit standard.

The mean value of these experiments, encumbered with an error of about 2 %, forms the basis for our definition of the unit, expressed in milligrams of the dry standard prepared from human serum.

$$1 \text{ unit} = 0.0809 \text{ milligram.}$$

We have thus secured a suitable amount of a stabile standard preparation which is adaptable to the assaying of human sera. This antistreptolysin standard will be stored in the same way as the internationally adopted standard antitoxins entrusted to the department, and solutions of the standard, having an accurately defined potency, is at the disposal of all laboratories interested.

The Practical Procedure Involved in the Assaying of Antistreptolysin for the Purpose of Diagnosis.

When assaying a serum of unknown potency the serum is measured off in diminishing doses and a constant weight of toxin is added. At the same time a similar series is prepared with the standard serum, and, upon completion of the process of hemolysis, a dilution of the serum will be found which gives the same degree of reaction as a known amount of the standard serum, expressed in units. Multiplying the degree of dilution with this number of units gives the contents of units per cc of the serum assayed.

This is a universal principle which is always applied to the assaying of sera, regardless of whether the reaction against the toxin is hemolysis, death or a skin reaction. It is a condition, however, for the use of this method that the reaction curves for the standard and the serum are parallel, or, as it also may be expressed, that similarly acting doses of the two preparations are proportional.

When assaying antistreptolysin in human sera this condition is only present when the standard is of the same quality as the serum. In practice we nevertheless reach very serviceable results by means of the rabbit standard, but it does give more security to apply a standard of human serum. —

Kalbak has described the details of the actual assay, but it has been found that by changing a few details it is possible to simplify the titration and the reading so that a routine investigation of a large number of samples is facilitated. In the following we shall give a complete description of a titration with these modifications incorporated.

Table II.
Scheme for Titration of Control Series.

	Dilution	Tube No.							
		1	2	3	4	5	6	7	8
Standard 1 cc 10 U. Lysin	(1+1)	0.20	0.16	0.125	0.10	0.08	0.064	0.05	0.04
	1 Test dose in 2 cc	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0
Units		1.0	0.8	0.64	0.5	0.4	0.32	0.25	0.20

Table III.
Scheme for Titration of unknown Serum.

	1. Dilution	Tube No.										
		1	2	3	2. Dilution	4	5	6	3. Dilution	7	8	9
Serum Lysin	(0.1+0.9)a	0.20	0.10	0.05	(0.2a+1.4)b	0.2	0.10	0.05	(0.2b+1.4)	0.20	0.10	0.05
	1 Test dose in 2 cc	2.0	2.0	2.0		2.0	2.0	2.0		2.0	2.0	2.0
Degree of Dilution		50	100	200		400	800	1600		3200	6400	12800

According to the titration schedules in tables II and III only two portions of liquid are to be added to each test tube¹⁾ instead of three as previously recommended, the volume of serum added being so small in proportion to the volume of the constant lysin amount that it is unnecessary to add saline solution to obtain the same volume in all test tubes.

The *standard serum*, which is prepared with 10 units per 1 cc, is diluted 1:2, 1 cc of the standard being added to 1 cc of buffer saline

¹⁾ Dwarf test tubes (70 × 10—11 mm), so-called »Widal tubes« are used.

solution¹). The dilution is measured off by means of a 0.20 cc capillary »delivery« pipette having 0.01 cc graduation.

The serum is diluted several times. Nine test tubes are placed in a rack with a dilution tube for every three of them, the first one of these dilution tubes containing 0.9 cc of buffer saline solution and the next two 1.4 cc of the same solution. The capillary pipette is now filled with serum to the 0.20 cc mark, and of this serum enough is let out again to lower the surface to the 0.09 cc mark. The pipette is now dipped into the first dilution tube and saline solution is sucked back and forth a couple of times all the way up to the 0.20 cc mark. This causes the rinsing out of 0.01 cc of serum which adheres to the wall of the capillary and there will be a total of 0.10 cc of serum in the dilution tube²). The three doses in test tubes 1, 2 and 3 are now measured off with the first serum dilution. Next the pipette is filled to the 0.20 cc mark and enough of the serum dilution is let out to lower the surface to the 0.19 cc mark. The pipette is transferred to the second dilution tube and rinsed to the 0.20 cc mark. After measuring off the second serum dilution in test tubes 4, 5 and 6 the operation is continued with the third dilution.

In routine assays, however, it does not pay to include more than the first 6 test tubes. If the potency of the serum exceeds 800 units it is then necessary to repeat the assay with the last 6 tubes, but such cases are rare, and it may then be advisable to check this strongly increased titer by repeating the assay.

The *lysin* is assayed once for all before the titration so that we know the dose that gives beginning reaction (hemolysis) mixed with 0.5 units, but giving no reaction with 0.63 units. If this test dose is 0.20 cc, for example, a dilution is prepared which in 2 cc contains 0.20 cc of lysin. The dilution can be made by means of a measuring cylinder since it always will be large quantities that are used for titration of a large number of sera. Thus if 40 sera are to be assayed it is necessary to prepare at $(40 \times 6 \times 2 + 8 \times 2) = 496$ cc of dilution. In that case we mix, say, 60 cc of lysin with 540 cc of buffer.

The lysin preparation is added to all test tubes by means of a large 10 cc pipette, calibrated to $\frac{1}{10}$ cc, or a corresponding burette.

The combination of lysin-antilysin occurs by letting the test tubes stand for $\frac{1}{4}$ hour in water bath (37°).

Rabbit blood corpuscles are then added from a 5 % suspension of washed blood corpuscles. 0.5 cc is added to each test tube. The tubes are turned and placed in the water bath for $\frac{3}{4}$ hour.

The reading of the degree of hemolysis may take place immediately after, the test tubes being centrifuged for 1 minute at 2000 r.p.m. he

¹) 14.526 gr. KH_2PO_4 , 76.006 gr. Na_2HPO_4 , 2 H_2O and 48.0 gr. NaCl in 10 liter redistilled water.

²) This use of a »delivery« pipette as a »containing« pipette has been introduced at the State Serum Institute by Dr. *Martin Kristensen*.

handled. It is found that by placing the racks for 2 *hours* at room temperature the sedimentation of the blood corpuscles is sufficiently complete to make it possible to judge the degree of hemolysis in the supernatant fluid. Centrifuging is necessary only if it is desired to obtain an accurate colorimetric reading. Comparison of a reading after immediate centrifuging and after a couple of hours' standing has not shown any difference in the results.

Calculation of the titer. The standard series is so adjusted that

		SERIES OF SERUM TO BE TESTED														
SERIES OF STANDARD SERUM		DEGREE OF DILUTION	50	100	200	400	800	1600	3200	6400	12800					
	UNITS	TUBE No.	1	2	3	4	5	6	7	8	9					
	1.0	1	50	100	200	400	800	1600	3200	6400	12500					
			45	90	180	360	700	1400	2800	5600	11000					
	0.8	2	40	80	160	320	640	1250	2500	5000	10000					
			36	70	140	280	560	1100	2200	4500	9000					
	0.64	3	32	64	125	250	500	1000	2000	4000	8000					
			28	56	110	220	450	900	1800	3600	7000					
	0.50	4	25	36	50	70	100	140	200	280	400	560	800	1600	3200	6400
			22	32	45	64	90	125	180	250	360	500	700	1400	2800	5600
	0.40	5	20	28	40	56	80	110	160	220	320	450	640	1250	2500	5000
18			25	36	50	70	100	140	200	280	400	560	1100	2200	4500	
0.32	6	16	22	32	45	64	90	125	180	250	360	500	1000	2000	4000	
		14	20	28	40	56	80	110	160	220	320	450	900	1800	3600	
0.25	7	12	18	25	36	50	70	100	140	200	280	400	800	1600	3200	
		11	16	22	32	45	64	90	125	180	250	360	700	1400	2800	
0.20	8	10	20	40	80	160	320	640	1250	2500						

the middle test tubes show different degrees of partial hemolysis, while as a rule only one tube in the serum series shows partial hemolysis. This test tube is then compared with the standard series and we find the tube in that series which shows the same degree of hemolysis, or the two tubes between which the degree of hemolysis of the serum sample lies. As mentioned above, the titer is found by multiplying the degree of dilution in the serum test tube with the number of units in the corresponding standard test tube. The attached schedule may serve to simplify the calculation, giving the titer directly as the figure at the intersection of the vertical column and the horizontal row belonging to the two test tubes in question.

If the serum series contains two tubes with partial hemolysis, or tubes with only total- and non-hemolysed contents, two readings are obtained. The mean of these two readings may then be found in the table by laying a ruler to connect the two titers in their adjacent columns, the mean being read in the column of small figures at the point where the ruler strikes this column.

The use of the titers given in the schedule insures a uniform titer

designation with values that deviate just so much from one another as one might expect from the accuracy of measurement, and the mean is in this way calculated as a geometric mean which, as in all computations involving antitoxin titers, is the most correct.

Summary.

For international use in the assaying of antistreptolysin O in human serum a stabile dry preparation of human serum has been produced. By comparison with *Todd's* standard serum (rabbit) 0.081 milligram of this serum is found to be equivalent to the unit for antistreptolysin defined by *Todd*. A clear comparison with antistreptolysin from man and rabbit is made difficult by a qualitative difference between the reaction curves for the two antitoxins. Hence a special technique is employed in the calculation of the potency of the human standard serum.

The technique of serial investigations of human sera is described, and a table presented for use in the practical calculation of the titer.

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RECHERCHES SUR LA FERMENTATION MUTATIVE DES BACTÉRIES

(4^e COMMUNICATION)

Par *Martin Kristensen*.

(Reçu par la Rédaction le 29. 8. 1943).

Jusqu'ici nous nous sommes principalement occupés du pouvoir mutatif des bactéries envers un seul sucre; pourtant on a mentionné au chapitre VII une expérience où l'on a fait continuer la mutation d'un bacille typhique, déjà muté envers un certain sucre (le xylose), envers un autre sucre (la dulcité). Ci-dessous on va rendre compte de quelques expériences systématiques, visant à produire, en partant d'une seule souche typhique, une série de cultures dont on décide d'avance les combinaisons de réactions envers la dulcité (Du), l'arabinose (Ar), le xylose (Xy) et le rhamnose (Rh).

La plupart des expériences montrent que la mutation envers un sucre donné ne comporte aucune modification des pouvoirs fermentatifs envers d'autres sucres, ou qu'il s'agit d'un pouvoir fermentatif typique ou du pouvoir mutatif. C'est ainsi que *Saisawa* a fait muter d'abord le bacille typhique envers la seule Du, le seul Ar, et le seul Rh; en faisant continuer la mutation d'une souche déjà mutée, envers un des autres sucres, on a produit des cultures qui ont fermenté respectivement la Du et l'Ar, la Du et le Rh, et l'Ar et le Rh; en faisant muter une culture, qui fermentait deux des trois sucres, envers la troisième, on a fini par produire une forme qui fermentait les trois substances de manière typique. *Morishima* trouva que le bacille typhique mutait envers la seule Du, le seul Ar ou le seul Xy; aucune de ces mutations n'entraînait les autres. Avec une souche du bac. coli atypique, capable de muter envers le lactose, le maltose et le saccharose *Nungster* a fait des expériences analogues à celles de *Saisawa*.

Cependant on a fait aussi l'expérience que tout en mutant envers une certaine substance une bactérie acquiert le pouvoir de fermenter d'autres substances. *Andresen* trouva qu'une souche du bac. prodigiosus, mutant envers le lactose, acquerrait par là aussi le pouvoir de fermenter la mannite, le maltose et le saccharose. Selon l'expérience faite par *Klieneberger* une souche du bac. coli acquit le pouvoir fermentatif et envers l'arbutine et envers la salicine, quand on la fit muter en présence d'une de ces sub-

stances. *Audureau* faisait muter le *moraxella lwoffi* envers l'acide glutarique, l'acide glutamique et l'acide succinique, tirant de ses expériences avec les trois substances des résultats correspondant exactement à ceux de *Saisawa* et de *Nungester*. Mais en même temps elle a trouvé que par la mutation envers l'acide succinique il se produisit simultanément un pouvoir de pousser sur l'acide fumarique et sur l'acide l-malique, de même qu'il se produisait aussi, par la mutation faite en présence de l'une de ces dernières substances, un pouvoir de pousser sur chacune des deux autres.

Pour nos expériences nous nous sommes servis d'une culture unicellulaire (« 6 b ») (produite le 11 août 1941) de la souche typhique no. 6 mentionnée ci-dessus (vol. 19, p. 551). De celle-ci on avait produit le 15 août 1941 une variante (« bleu-blanchâtre ») à croissance non entravée sur xylose (« 6 b n »). Ces deux formes ont été conservées comme des cultures en piqure dans la gélose d'extrait de viande; on les aensemencées dans le même milieu le 20 février 1942. Le 28 février 1942 on aensemencé la forme non entravée dans un certain nombre de verres à Xy (expérience mentionné vol. 20, p. 547). D'un verre jauni le 18^e jour on a isolé une forme fermentant fortement le xylose (« 6 b n, b ») qui toute comme les autres a été conservée comme culture en piqure. Le 24 août 1942 toutes les trois formes ont étéensemencées dans le bouillon.

Pour abrégé on désignera ci-dessous la forme originale par la lettre O, (qu'il ne faut pas confondre avec la forme sérologique O) la forme non entravée, bleu blanchâtre par (Xy) et le mutant fermentant le Xy par Xy. On se servira de qualifications correspondantes pour les mutants envers la Du, l'Ar et le Rh, à savoir respectivement Du, Ar et (Rh). La parenthèse indique que la mutation consiste seulement à surmonter l'entrave, non pas dans l'acquisition d'un pouvoir fermentatif. Le résultat des mutations combinées est marqué par une combinaison de ces expressions, de sorte que XyArDu(Rh) p. ex. indique une culture qui par une mutation successive envers le Xy, l'Ar, la Du et le Rh dans l'ordre signalé a acquis le pouvoir de fermenter les trois sucres avec acidification et de pousser sans entrave dans Rh.

Pour produire des formes mutées on a commencé par ensemencer dans un verre à bouillon ou d'une piqure en gélose ou d'une colonie se trouvant au stade de mutation immédiatement précédent; du verre à bouillon on aensemencé un bouillon d'extrait de viande (ExB), renfermant 0,4 % ou 0,5 % de l'espèce de sucre envers laquelle on désirait faire muter la culture; la fermentation survenue on aensemencé une plaque à peptone Parke-Davis, mentionné ci-dessus (vol. 19, p. 552), renfermant ½ % du même sucre. Dans trois cas on a réensemencé de nouveau de la colonie jaune dans l'ExB avec la même espèce de sucre (Du) et de là on a fait une nouvelle dissémination; dans deux cas on a commencé par faire un ensemencement de la colonie jaune dans le bouillon, et de là on a piqué dans la gélose. Ces détails sont signalés dans le tableau ci-dessous, où se trouve indiqué aussi le volume de liquide nutritif dans lequel s'est produite la dernière mutation et le temps s'écoulant jusqu'au jaunissement du verre ou du flacon. Il est à remarquer

qu'on aensemencé de chaque variante ou un seul flacon, ou 5 verres; on n'a utilisé qu'un seul de ces verres pour l'ensemencement sur plaque.

Sauf indication contraire on a piqué directement en gélose de la colonie jaune.

Variante produite	c.c.	Jours	Intermédiaires entre la colonie et la piqure en gélose.
<i>Du</i>	Du 2	20	Du-ExB — plaque à Du
<i>Ar</i>	Ar 50	5	
<i>ArDu</i>	Du 2	45	
<i>(Xy)Du</i>	Du 2	15	
<i>(Xy)Ar</i>	Ar 50	2	bouillon
<i>(Xy)ArDu</i>	Du 2	23	
<i>XyDu</i>	Du 2	10	Du-ExB — plaque à Du
<i>XyAr</i>	Ar 50	3	bouillon
<i>XyArDu</i>	Du 2	7	Du-ExB — plaque à Du

Des 9 variantes ci-dessus et de leurs stades antérieurs *O*, *(Xy)* et *Xy* on a ensuiteensemencé dans ExB avec Rh; au bout de deux jours on a continué d'ensemencer de là dans du nouveau ExB à Rh; le lendemain on a disséminé des deux cultures liquides sur des plaques à Rh. Pour toutes 24 variantes il s'est produit ainsi une abondance de colonies non entravées, et, à ce qu'il semble, dans beaucoup de cas à l'état de pureté; la dissémination des cultures secondaires en Rh-ExB était en tout la plus pure. D'une colonie bien isolée de chacune des 12 formes on a piqué en gélose d'extrait de viande. On a donc produit tout méthodiquement ces 24 variantes (la forme originaire y comprise):

<i>O</i>	<i>(Xy)Du</i>	<i>(Rh)</i>	<i>(Xy)Du(Rh)</i>
<i>(Xy)</i>	<i>(Xy)Ar</i>	<i>(Xy)(Rh)</i>	<i>(Xy)Ar(Rh)</i>
<i>Xy</i>	<i>(Xy)ArDu</i>	<i>Xy(Rh)</i>	<i>(Xy)ArDu(Rh)</i>
<i>Du</i>	<i>XyDu</i>	<i>Du(Rh)</i>	<i>XyDu(Rh)</i>
<i>Ar</i>	<i>XyAr</i>	<i>Ar(Rh)</i>	<i>XyAr(Rh)</i>
<i>ArDu</i>	<i>XyArDu</i>	<i>ArDu(Rh)</i>	<i>XyArDu(Rh)</i>

Ensuite toutes les 24 variantes ont été ensemencées dans une série de milieux différents pour contrôler si leurs caractères correspondent aux formules indiquées. On aensemencé en ExB avec les espèces de sucre que voici: le lactose + le saccharose, la mannite, l'adonite, la Du, la sorbite, l'Ar, le Xy, le Rh, le maltose, la salicine; on a surveillé pendant 14 jours ou jusqu'à une réaction positive. En outre on a disséminé sur plaques respectivement de Du, d'Ar, de Xy, de Rh et sans sucre. On a fait ensuite une réaction d'indol, une microscopie avec coloration de Gram et une agglutination sur lame avec les sérums qui importent le plus au diagnostic du bacille typhique. Dans les milieux à sucres il s'est produit dans tous les cas des réactions caractéristiques pour le bacille typhique appartenant aux types mutatifs signalés. La réaction d'indol a été négative partout. En faisant des examens au microscope on a trouvé dans tous les cas des cultures pures de bacilles ne prenant pas le Gram. A l'agglutination sur lame toutes les variantes à l'exception de *DuRh* se comportèrent de la manière que voici: agglutination avec le sérum XXIX Vi z₁₄ (Ballerup

O Vi H) et le sérum d, aucune agglutination avec le sérum IV.V. Envers le sérum IX les cultures se sont comportées de manière différente. Le *Du(Rh)* a été agglutiné par les trois sérums premiers nommés, mais non pas par le sérum d. Il était rugueux et faiblement mobile, ce qui explique tant l'agglutination avec le sérum IV.V que le défaut d'agglutination avec le sérum d.

D'une importance particulière pour la caractérisation des variantes fut la dissémination sur plaques renfermant les quatre sucres; ici aussi les formules indiquées ont été complètement confirmées:

Sur la plaque à Du toutes les variantes dont la formule contenait *Du* prirent une coloration jaune ou jaune verdâtre au cours de 2 jours, tandis que les autres restèrent bleues.

Sur la plaque à Ar toutes les variantes dont la formule contenait *Ar* prirent une coloration intensément jaune au cours de 24 heures; toutes les autres restèrent bleues ou bleu verdâtre.

Sur la plaque à Xy toutes les variantes dont la formule contenait *Xy*, prirent une coloration jaune au cours de 24 heures; toutes les variantes dont la formule contenait (*Xy*) développèrent sans entrave des colonies bleues ou bleu blanchâtre; celles dont les formules ne contenaient pas *Xy* ou (*Xy*), développèrent des colonies verdâtres entravées, dans une partie desquelles à chaque dissémination des bourgeons blanchâtres parurent au cours de 48 heures.

Sur la plaque à Rh toutes les variantes dont la formule contenait (*Rh*) se développèrent sans entrave; les autres produisirent des colonies entravées dans une partie desquelles des bourgeons de la forme non entravée parurent dans chaque dissémination au cours de 48 heures.

Dans ce mémoire les mots de »bourgeon« et de »colonie secondaire« s'équivalent. De même les mots de »variante« et de »forme« sont employés indistinctement. On désigne par le mot de »modification« les cultures de la même variante, qui ne se distinguent que par la fréquence de mutation ou par d'autres particularités, ne modifiant pas la formule de mutation.

Les disséminations sur la plaque à Du des variantes *Xy*, *XyAr* et *XyAr(Rh)* consistaient en un mélange de colonies petites et grandes, où les grandes étaient en minorité. En comparant avec les cultures correspondantes sur les plaques sans sucre on a dû appeler « forme normale » les grandes colonies; les petites colonies « forme entravée dans sa croissance sous l'influence de la dulcité ». Des expériences ultérieures il ressortit que la forme *Xy(Rh)* se comportait de la même manière (ce qui vraisemblablement a été négligé la première fois); il s'agit donc de toutes les formes *Xy*, qui n'avaient pas muté envers Du avec acidification. Ces formes *Xy* seront l'objet d'une étude plus approfondie au chapitre IX.

Pour se faire une idée de la stabilité des pouvoirs fermentatifs acquis par les mutations réitérées on aensemencé la variante *XyArDu(Rh)* dans 5 verres d'ExB contenant respectivement la Du, l'Ar, le

Xy, le Rh et la sorbite; de là on a fait un ensemencement ultérieur dans de nouveaux verres avec la même espèce de sucre. De cette manière on a pratiqué au cours de 7 jours 4 passages en Xy et 7 passages dans chacune des autres espèces de sucre. Ensuite on a ensemencé tant la culture, qui n'avait pas subi ces passages, que les 5 cultures « de passage » sur des plaques de Du, d'Ar, de Xy, de Rh et sur des plaques sans addition. En comparant la manière de croissance des 6 cultures et le degré de coloration jaune de chacun des trois milieux renfermant la Du, l'Ar et le Xy on n'y a observé aucune différence entre elles, et la croissance sur la plaque à Rh était non entravée comparée à la croissance sur le milieu de base.

De toutes les expériences décrites il ressort: 1) que la survenue d'une mutation ne comporta aucune modification quant aux sucres que la souche fermente de manière typique, 2) que le pouvoir de muter envers les autres sucres ne fut pas supprimé, 3) qu'une souche qu'on fit successivement muter envers plusieurs espèces de sucre n'« oublia » pas les pouvoirs fermentatifs premièrement acquis, 4) que par les réensemencements réitérés dans chacune des espèces de sucre envers lesquelles les bactéries ont acquis le pouvoir fermentatif, il ne se produisit aucune adaptation spéciale à celle-ci.

Un nouveau contrôle de la stabilité des 24 variantes fut entrepris 7 mois e. après l'expérience principale. Chaque variante, qui avait été conservée en piqûre sans réensemencement fut ensemencée dans un tube de bouillon. Les tubes furent rénumérés par une aide, et ce n'est qu'après l'exécution de la classification des cultures que l'expérimentateur prit connaissance de la numération originelle des cultures. Les cultures en bouillon furent ensuite ensemencées sur des plaques à Du, Ar, Xy et Rh et sur des plaques sans addition. Selon l'aspect de ces cultures toutes les variantes furent classifiées sans ambiguïté et conformément à la classification originelle, à cette seule exception: dans la culture de la variante *XyDu* il y avait aussi des colonies du bacille typhique qui poussaient et sur le Xy et sur la Du sans fermentation et sans entrave; sur la plaque à Xy la culture était bleu verdâtre; la culture se comporta donc comme la forme *Xy'*. C'est à croire qu'il ne s'agit pas d'une contamination, mais d'une perte spontanée du pouvoir de fermentation envers les deux sucres.

A côté des 24 variantes principales il a été produit une série de modifications dont on va mentionner ci-dessous les particularités, puisqu'il en ressort qu'on n'obtient pas toujours des résultats aussi réguliers que décrit ci-dessous.

Dans la culture sur la plaque à Du, de laquelle on a isolé la variante *Du*, on a trouvé un mélange de grandes colonies (c. à d. de grandeur ordinaire) et de petites colonies. C'est une des grandes colonies qu'on a employée comme variante *Du* type et pour la production de la variante *Du(Rh)* type. En disséminant sur une plaque à Du d'une grande colonie on a obtenu uniquement de grandes colonies; en disséminant d'une petite colonie on a eu uniquement

de petites colonies qui cependant étaient de deux grandeurs différentes. Une des plus grandes parmi celles-ci a été employée pour la production des variantes *DuAr*, *DuArXy*, *Du(Rh)*, *DuAr(Rh)* et *DuArXy(Rh)*. (A l'exception du *Du(Rh)* les mutations se sont donc produites dans un autre ordre que dans la série type). Par dissémination sur une plaque sans sucre toutes ces variantes ont donné des colonies relativement petites; pour la forme *Du* il y eut aussi un nombre restreint de grandes colonies. Dans ces cas la croissance entravée observée au début sur la plaque à *Du* n'est donc pas due à un effet spécial de la dulcité.

Sur les plaques à *Du* l'acidification des 6 variantes se fit assez lentement. Elle était le plus vif dans *Du* et *Du(Rh)*, le plus faible dans *DuArXy* et *DuArXy(Rh)*.

Sur la plaque à *Ar* la réaction était conforme aux formules et sans particularités; dans la culture *DuArXy* sur ce milieu on a observé un mélange de colonies d'un jaune plus ou moins clair.

Sur la plaque à *Xy*, *Du* et *Du(Rh)* commencèrent par donner une croissance verdâtre entravée, mais au cours de 2 ou 3 jours ils se sont teints en bleu sans que des bourgeons s'y soient produits (quant à leur comportement vers le *Xy*, ces formes sont donc des intermédiaires entre 0 et $\langle Xy \rangle$); par contre il y eut le 2^e jour une formation typique de bourgeons dans le *DuAr* et le *DuAr(Rh)*. Dans *DuArXy* et *DuArXy(Rh)* les colonies sont devenues purement jaunes au bout de 2 jours.

Sur la plaque à *Rh* *Du*, *DuAr* et *DuArXy* ont poussé entravés avec des petits bourgeons le 3^e jour; les trois autres ont poussé non entravés, mais dans la culture *DuArXy(Rh)* des bourgeons ont paru le 2^e jour; à des recherches répétées elle a poussé entravée sur *Rh*, avec des bourgeons le 3^e jour. Il ne s'agit donc pas d'un mutant *Rh* typique. Plus tard on a obtenu une nouvelle variante *DuArXy(Rh)* à partir de la forme *Du* à croissance faible; cette fois-ci la croissance sur la plaque à *Rh* était typiquement non entravée, et au début et à un examen après 3 mois de repos comme culture en piqure.

Sur lame toutes les souches ont été agglutinées par les sérums IX et XXIX Vi₂₁₄; mais à l'exception de *DuArRh* elles ont été agglutinées aussi par le sérum IV.V; le *DuArXy*, le *DuAr(Rh)* et le *DuArXy(Rh)* n'ont pas été agglutinés par le sérum d.

Il s'agit donc d'une série de variantes entravées à l'égard de l'énergie de croissance, atypiques au point de vue sérologique (rugueuses?), et qui en partie ont présenté aussi des particularités atypiques lors des mutations fermentatives impropres. Ici encore les mutations proprement dites (c. à d. avec acidification) se sont comportées de la même manière que dans la série principale.

IX. Rapports du mutant de *Xy* à l'égard de la *Du*.

En étudiant de plus près les variantes *Xy*, *XyAr*, *Xy(Rh)* et *XyAr(Rh)* on a trouvé qu'elles se présentent sous deux formes: une forme primaire qui faisait fermenter le *Xy* (colonies jaunes sur plaque à *Xy*) et poussait entravé sur plaque à *Du*, et une forme secondaire, poussant avec des colonies vertes ou bleu verdâtre¹⁾ sur plaque à *Xy* et avec des colonies bleues non entravées sur plaque à *Du*. Donc, la forme *Xy* perd le pouvoir de fermenter le *Xy* en même temps que l'entrave sur

la plaque à Du est surmontée. Nous appellerons Xy' la nouvelle forme obtenue par ce procédé²⁾; elle se produit par une mutation qui peut s'effectuer spontanément, c. à d. sans que la culture entre en contact avec le Xy ou la Du; mais la croissance du mutant comparé à celle de la forme primaire est naturellement fortement favorisée par la présence de la Du. La couleur de la végétation sur la plaque à Xy ressemble à celle de la forme O , mais la croissance est plutôt non entravée, et il y a, au plus, une disposition faible à former des bourgeons au cours des deux premiers jours; après quoi il peut se produire des bourgeons jaunes.

Sur la plaque à Du la forme Xy peut former, même au bout de 2 ou 3 jours, des bourgeons jaunes, et ce mutant Du fait toujours fermenter le Xy comme il ressortait déjà de la production de la forme $XyDu$ dans la série principale. Il paraît tout indiqué de présumer que c'est la forme Xy (à savoir la forme entravée sur la plaque à Du) qui mute directement dans la forme $XyDu$, tout en conservant le pouvoir fermentatif envers le Xy. Comme nous le verrons ci-dessous la forme Xy' peut muter aussi envers la Du, ce qui cependant se produit plus difficilement.

Les expériences ci-mentionnées ont été faites tant avec les variantes originaires qu'avec les cultures unicellulaires des formes Xy , $XyAr$ et $Xy(Rh)$.

Ensuite on a produit 7 cultures unicellulaires de la forme Xy' , lesquelles ont été étudiées quant à leur pouvoir de muter envers le Xy et la Du. 3 des cultures ont été ensemencées chacune dans 8 verres à Xy, les 4 autres dans un seul verre à Xy chacune. Dans 26 de ces 27 verres un jaunissement est survenu au cours de 5 à 8 jours; de 9 des verres on a ensemencé sur une plaque à Xy, par où (en dehors de colonies bleues) des colonies jaunes se sont produites dans tous les cas. Sur plaque à Du la forme jaune ainsi obtenue se comportait tout à fait comme la forme Xy originale, c. à d. qu'elle poussait entravée, mais avec des colonies bleues secondaires le 3^e jour; un ensemencement sur des plaques à Xy d'une colonie entravée donna des colonies jaunes, tandis qu'une colonie bleue secondaire lors d'un ensemencement continué sur une plaque à Xy poussa avec une coloration verte.

Donc: Xy sur Du $\longrightarrow Xy'$ (ou $XyDu$)
 Xy' sur Xy $\longrightarrow Xy$
 Xy sur Du $\longrightarrow Xy'$

....

¹⁾ La couleur peut se transformer en bleue à force d'une culture prolongée; en dissimulant une culture sur plaque à Xy on peut observer un mélange de colonies bleu verdâtre et bleu blanchâtre. Par des titrages exécutés d'après *Hagedorn-Schmidt* nous avons vérifié que le pouvoir fermentatif de la forme Xy' envers le Xy est nulle ou très faible.

²⁾ Si nous faisons abstraction de l'origine de cette forme et de sa couleur verdâtre nous pouvons aussi la classer sous la désignation de (Xy) .

3 des cultures unicellulaires de la forme Xy' ont été ensemencées chacune dans 16 verres à Du. Pendant 60 jours de surveillance une coloration jaune ne s'est produite que dans 2 des 48 verres. L'un des verres jaunît partiellement le 49^e jour, complètement le 50^e jour, l'autre partiellement le 50^e jour, complètement le 52^e jour. En disséminant sur des plaques à Du il s'est produit dans les deux cas un mélange de colonies bleuies et jaunes. De chacune des plaques on a continué la culture des 4 colonies jaunes, de la première plaque en outre d'une colonie légèrement jaune et d'une colonie bleue, de l'autre plaque de deux colonies bleues. A une agglutination sur lame toutes les 12 colonies ont été agglutinées par le sérum XXIX Vi z_{14} et par le sérum d, mais non pas par le sérum IV. V, ni par le sérum IX; elles se sont donc comportées comme des bacilles typhiques typiques de la forme V. 8 colonies jaunes typiques ont donné des colonies jaunes lors d'un ensemencement sur des plaques à Xy; cependant 4 d'entré elles ont pu dissocier une forme, qui poussa en de petites colonies sur plaque à Du, et qui ne fit fermenter ni la Du, ni le Xy de manière typique, mais qui produisit des bourgeons jaunes sur la plaque à Xy. Il s'agit donc du même phénomène que celui décrit au chapitre VIII: perte simultanée du pouvoir fermentatif envers la Du et le Xy.

Le résultat principal des expériences sur la mutation de la Du, décrites ci-dessus, pourra s'exprimer par la formule:

$$Xy' \text{ sur Du} \longrightarrow XyDu$$

qui énonce qu'en faisant muter la forme Xy' envers la Du on peut lui faire recouvrer son pouvoir fermentatif envers le Xy.

Toutes les mutations envers la Du et le Xy observées peuvent se résumer dans la figure 7.

La mutation $(Xy)Du \longrightarrow XyDu$ par la culture dans le milieu à Xy fut démontrée par une expérience supplémentaire. Il y en avait deux modifications, se distinguant nettement par l'intensité de l'acidification sur plaque à Xy et sur plaque à Du. Cependant, à côté de ce mutant typique on trouva aussi une forme Xy qui fermentait le Xy, mais non pas la Du; sur la plaque à Du elle était entravée avec formation précoce de bourgeons bleu blanchâtre; les subcultures de ces bourgeons poussaient sur plaque à Xy sans fermentation et sans entrave; leur couleur variait entre le bleu et le bleu vert (forme Xy').

Pour le coup ces deux questions se posent: les conditions de croissance et de fermentation sur des plaques de Xy et de Du, vérifiées pour la souche étudiée, sont-elles valables pour d'autres souches typhiques du type 2 aussi? sont-elles valables pour la forme Xy naturelle, c. à d. les bacilles typhiques du type 1 (et du type 3, par où l'on comprend la forme naturelle $XyAr$ très rare)?

Pour jeter du jour sur ces questions on a choisi dans les collections

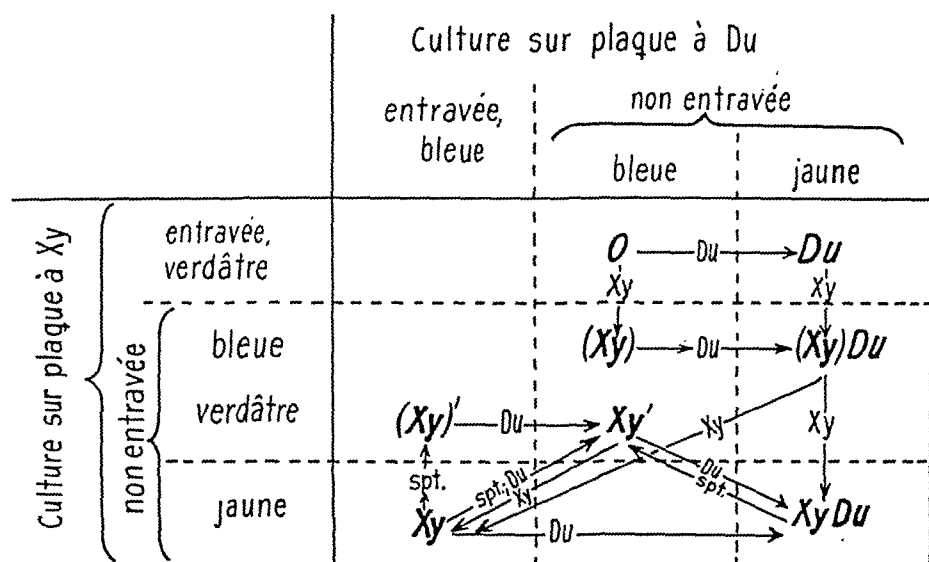


Fig. 7.

Les mutations du bacille typhique envers la dulcité et le xylose. Les flèches indiquent les directions des mutations. »Du« ou »Xy« dans une flèche indique le milieu sur lequel la mutation fut observée.

»spt.« = spontanément, c. à d. que la mutation fut observée sur le milieu sans sucre.

de l'Institut 41 souches du type 1, 18 souches du type 2 et une souche du type 3.

Des souches du type 1 7 proviennent du Central International de Salmonella; les autres avaient été isolées d'échantillons de matières fécales, d'urine et de sang, remis au cours des années 1941—42. La souche no. 9 du type 2 a été isolée il y a plus de 40 ans par C. O. Jensen (sous la désignation de T 9); il en est de même pour la souche du type 3 (T 5). Parmi les autres souches du type 2 8 sont égales aux souches 1—8 déjà étudiées; les numéros 10—17 et 19 ont été isolées au cours des années 1940—42 (le no. 12 d'urine, le no. 19 de sang, les autres de matières fécales).

Les souches isolées à l'Institut même ont été choisies de manière à ce qu'il n'y eût qu'une seule de chaque malade et d'ordinaire qu'une seule de la même partie du pays.

Toutes les souches des types 1 et 3 ont poussé sur la plaque à Xy uniquement avec des colonies jaunes; sur la plaque à Du elles poussaient tout aussi bien que sur le seul milieu de base (une d'entre elles mieux sur la plaque à Du).

Dans 14 des souches du type 1 il y eut et sur la plaque à Du et sur le milieu de base un mélange de colonies plus ou moins grandes, tandis que dans les autres souches les colonies furent de grandeur à peu près égale.

Donc la forme *Xy naturelle* n'est pas entravée par la Du.

Parmi les souches du type 2 deux existèrent déjà sous forme d'une ou de plusieurs cultures en piqure en gélose d'extrait de viande de la forme *Xy*.

Parmi celles-ci les cultures marquées de 6.4 et de 8.4 dataient d'environ 15 mois, celles marquées de 6bn... de 9 mois.

Voici comment se comportèrent ces cultures:

	Couleur sur plaque à Xy		Croissance sur plaque à Du
	Originairement	à de nouveaux examens	
6.4	jaune	jaune	colonies entravées et non entravées (e. 1000 : 1)
6bn ₁ a	légèrement jaune	légèrement jaune	do.
6bn ₆ a	légèrement jaune	légèrement jaune	entravée, avec des bourgeons le 2 ^e jour
6bn ₆ b	fortement jaune	colonies bleues et jaunes	do.
8.4	jaune	acidification douteuse, croissance un peu entravée	do.

Donc, 6.4, 6bn₁a et 6bn₆a se comportèrent de même que 6bn₆a produite en même temps (forme (Xy) de la série principale). De 6bn₆b on aensemencé des colonies bleues et jaunes sur une plaque à Du; dans les deux cas il s'est produit une croissance entravée avec formation de bourgeons. De la croissance entravée ainsi que des bourgeons on a réensemencé sur une plaque à Xy. Ainsi la croissance entravée de 6bn₆b »jaune« a donné des colonies jaunes, tandis que les cultures provenant des bourgeons ont poussé teintes en bleu. De 6bn₆b »bleu« et la croissance entravée et les bourgeons ont donné une croissance bleue sur une plaque de Xy. Nous désignerons comme (Xy)' la forme qui »spontanément« avait perdu son pouvoir fermentatif envers le Xy avec conservation de son comportement envers la Du.

Les souches non mutées du type 2 poussaient toutes sur une plaque à Xy avec des colonies entravées et verdâtres. Le 3^e jour la souche no 1 donna nombre de bourgeons jaunes; le no 19 n'a produit aucuns bourgeons en 6 jours; dans les autres cultures on a vu paraître des bourgeons blanchâtres du 2^e jusqu'au 4^e jour. Sur la plaque à Du les souches se comportèrent absolument comme la souche du type 1, poussant également bien sur des plaques avec et sans Du. Sur la plaque à Du 7 d'entre elles donnèrent des colonies de grandeur variable, mais il en était de même sur le milieu exempt de Du.

De toutes les 16 souches on a par culture dans XyExB produit la forme Xy, et celle-ci a été ensemencé sur une plaque à Xy, une plaque à Du et sur une plaque sans addition aucune. Sur la plaque à Xy la souche 17 a donné un mélange de colonies jaunes et vertes; les autres souches ont donné uniquement des colonies jaunes, pourtant dans quelquesunes des souches la coloration jaune n'était qu'assez légère.

Les souches 1 et 2 (qui comme nous l'avons mentionné vol. 19 p. 551 et 554 étaient originaires des îles Féroé et possédaient un pouvoir fermentatif primaire relativement fort) donnèrent sur la plaque à Du une croissance non entravée et égale; donc, ces deux souches se comportèrent sur la Du comme le type 1.

Les souches 9 et 19 donnèrent sur la plaque à Du (non pas sur le milieu de base) des colonies de grandeur variable, sans distinction précise entre une forme entravée et une forme non entravée. En réensemencant sur le Xy des plus grandes colonies le no. 9 n'a donné rien que des colonies jaunes, mais à deux intensités différentes de couleur; le no. 19 a donné une croissance à une acidification faible semblable à celle de la culture Xy originaire. Ici non plus il n'y eut question d'une transformation $Xy \rightarrow Xy'$. Sur la plaque à Du les autres cultures ont donné une croissance entravée à formation de bourgeons du 1^{er} jusqu'au 3^e jour. En réensemencant des bourgeons sur une plaque à Xy les nos. 3, 4, 7 et 17 ont donné une croissance bleue, les autres une croissance verte, partie avec formation de bourgeons jaunes ou de bourgeons blanchâtres.

Dans les cultures sur plaque à Du des souches 3, 4 et 7 des bourgeons jaunes ont apparu en dehors des bourgeons blanchâtres déjà mentionnés; en réensemencant de celles-ci sur une plaque à Xy une croissance jaune s'est produite conformément aux expériences faites avec la souche 6.

En résumé brièvement ce que nous venons de dire voici comment on pourrait classer les souches:

Aucune entrave ou entrave non caractéristique sur plaque à Du, conservation du pouvoir fermentatif envers le Xy: 1, 2, 9, 19.

Entrave et ensuite mutation sur la Du; le mutant pousse sur une plaque à Xy a) avec coloration verte: 5, 10, 11, 12, 13, 14, 15, 16, b) avec coloration bleue: 3, 4, 7, 17.

X. Observations concernant la mutation de Rh et d'Ar.

Les expériences qu'on va traiter dans ce chapitre on aurait pu les mentionner avant le chapitre sur les mutations combinées; si ce n'est qu'ici qu'on les mentionne, c'est que les recherches sur la mutation Rh ($O \rightarrow (Rh)$) traitent entre autres choses de la question d'un rapport entre celui-ci et la mutation correspondante envers le Xy ($O \rightarrow (Xy)$); et les conditions de la mutation d'Ar pure sont d'un certain intérêt pour les expériences qu'on va rapporter dans le chapitre XI.

Trois souches du type 1 (F 17136/41—42, F 17807/41—42, et F 18640/41—42) chacune de sa région du pays, et une culture unicellulaire de chacune des souches 1, 2, 3, 5, 6 et 7 du type 2 ont été ensemencées dans des milieux liquide et solide à Rh. Dans tous les cas une mutation se produisit ou dans le milieu liquide ou dans le milieu solide ou dans les deux. On isola la forme mutée pour la disséminer ensuite sur une nouvelle plaque à Rh; dans aucun cas des bourgeons ne se produisirent à 8 jours de surveillance.

On a ensuite fait des expériences sur la question de savoir si la mutation était accompagnée d'un pouvoir d'utiliser le Rh. Pour cela on employa tant les souches originaires que les souches mutées à

l'exception des nos. 1 et 3; pour la recherche de l'action réductive et le comptage des bactéries on n'a pourtant pas employé toutes les 7 souches restantes.

1. Tant la forme originale que la forme mutée ont été ensemencées en liquide nutritif (2 % de bactopectone) et sur le milieu solide ordinaire, toutes les deux avec et sans Rh. Pour toutes les 7 souches la culture originale était entravée sur plaque à Rh alors que les trois autres cultures — la culture originale sur plaque sans Rh la culture mutée sur plaque avec et sans Rh — se comportèrent de manière à peu près égale. Donc la mutation de Rh était sans influence sur la croissance en milieu exempt de Rh, mais eut pour effet que la croissance n'était plus entravée par le Rh. Dans les milieux liquides la culture présenta des particularités correspondantes sauf que le mutant de Rh de la souche F 17807 était toujours quelque peu entravé comparé tant à la forme originale qu'à la forme mutée dans le milieu exempt de Rh, mais non pas si fortement que la forme originale dans le même milieu renfermant Rh. Par contre on n'a dans aucun cas observé des signes que le Rh augmentât la croissance.

2. Dans aucun cas il ne s'est produit une acidification, ni sur le milieu solide, ni dans le milieu liquide. Il y a ici une différence des rapports à l'égard du Xy, où la forme originale donna une acidification faible.

3. Dans aucun cas on n'a observé une réaction positive dans un milieu liquide de Rh avec fuchsine, décolorée à l'aide de sulfite de sodium, comme indicateur.

4. On pourrait espérer d'obtenir de l'intensité de la croissance une estimation plus exacte par comptage des bactéries qu'en observant la grandeur des colonies et le degré du trouble. On a fait un comptage vital en appliquant la même technique qu'auparavant lors d'un ensemencement de culture dans des milieux exempt de Rh et renfermant le Rh.

Tableau 11.

Comptages vitaux de cultures de la forme (Rh) dans milieu sans et avec Rh.

				Age de la culture		
Souche no.	7	sans Rh avec »		8 jours	35 jours	39 jours
				221	45	73
				227	68	24
»	»	8	sans »	274	23	18
			avec »	81	45	18
»	»	17807	sans »	465		
			avec »	76		
»	»	18640	sans »	64	52	66
			avec »	99	29	32

Les chiffres indiquent le nombre de millions de bactéries par cc.

Les chiffres du tableau même indiquent les millions de bactéries par c.c.

Donc, dans la plupart des cas il y avait tout de même une croissance moins forte dans le milieu renfermant le Rh que dans le milieu exempt de Rh; dans les cas où le nombre des colonies était le plus grand lors de l'ensemencement provenant du milieu à Rh, la différence se montra inconstante à des recherches répétées.

5. Apparemment l'épreuve par la réduction à laquelle nous avons soumis la culture de la forme mutée dans un milieu à Rh montra en moyenne une consommation très restreinte de Rh, mais le résultat est peu sûr, la variabilité étant trop grande par suite de ce que l'action réductrice était loin d'être terminée au bout des 15 minutes d'ébullition.

Il ressort des expériences décrites sous 1—5 que dans plusieurs cas (vraisemblablement assez souvent) le mutant (*Rh*) est encore un peu entravé par la Rh, par contre il est très incertain s'il acquiert le pouvoir d'utiliser la Rh.

Pour la recherche sur la possibilité d'un rapport entre les réactions analogues $0 \longrightarrow (Rh)$ et $0 \longrightarrow (Xy)$ on a employé 25 cultures en gélose d'extrait de viande, qui plus ou moins de temps auparavant avaient été isolées de cultures unicellulaires des souches 2, 5, 6, 7 et 8.

De celles-ci 5 n'avaient pas été cultivées auparavant en Xy ou en Rh. En disséminant sur des plaques à Xy, des plaques à Rh et sur une plaque sans addition elles se trouvèrent toutes entravées tant par le Xy que par la Rh.

Selon des expériences antérieures 11 cultures avaient muté envers le Xy (dans la forme (*Xy*)), mais non pas envers le Rh. Elles se montrèrent toutes entravées par le Rh. 8 d'entre elles continuèrent leur croissance non entravée sur le Xy, tandis que 3 autres devaient être qualifiées de formes transitoires entre la forme originaire et la forme non entravée; donc, ces trois cultures (elles avaient été conservées comme culture en piqure pendant 9 mois) avaient subi une action en retour partielle.

Selon des expériences antérieures 9 cultures avaient muté envers le Rh, mais n'avaient pas passé par le Xy; elles poussaient toutes non entravées sur la Rh, entravées sur le Xy.

Déjà du chapitre VIII il ressortit que les mutations envers le Xy et le Rh peuvent se produire indépendamment l'une de l'autre; les expériences ci-exposées démontrent la même chose d'une manière plus détaillée.

Le cours de la mutation envers l'Ar n'a pas été étudiée si minutieusement que le cours de la mutation envers la Du et le Xy. Les expériences faites jusqu'ici portaient à croire que la mutation envers l'Ar se comporte d'une manière particulière, en ce qu'un petit nombre des cultures (en verres de 2 c.c. e.) mutant au cours des 2 ou 3 premiers jours avec une couleur jaune intense, alors que des mutations

tardives étaient d'une occurrence beaucoup plus rare. Pour en faire une étude plus approfondie on aensemencé la souche typhique 6 dans 96 verres d'Ar. Au cours des 66 premiers jours 18 verres se sont teints en jaune aux époques que voici: 2, 2, 3, 3, 6, 9, 11, 13, 13, 15, 25, 26, 40, 44, 46, 48, 52 et 66 jours après l'ensemencement. A une représentation graphique des résultats de la méthode antérieurement appliquée la courbe présente une chute beaucoup plus forte dans les 3 premiers jours que pendant la partie ultérieure du cours. Pour les mutations les plus tardives on s'est assuré, en examinant au microscope le verre même, la dissémination et l'agglutination des colonies bleues et jaunes, que la fermentation n'est pas due à des souillures. Les cultures pures des mutants ultérieurs ont fermenté assez tardivement, tandis que les mutants précoces (d'après d'autres expériences différentes) donnèrent justement des colonies intensément acidifiantes; sur ce point, mais non pas quant au cours de la courbe de mutation, le bacille typhique se comporte donc comme *Salmonella dublin*.

XI. *Les bactéries fermentaires proviennent-ils d'un seul ou de plusieurs individus de la culture originale?*

Les idées ordinaires sur la nature de la réaction mutative qui ont fait jusqu'ici la base de nos études comportent que c'est un seul individu bactérien qui mute, après quoi le mutant vu ses meilleures conditions nutritives se développe avec beaucoup plus de force que la partie non mutée de la culture; même si au bout de quelque temps la plus grande partie des bactéries d'un verre sont du type muté ceci n'empêche donc pas que la mutation même ne s'est faite valoir que dans un seul individu bactérien. Sans doute, dans une culture provenant d'une seule souche, dont la fréquence mutative est grande, trouve-t-on souvent plusieurs mutants indépendants l'un de l'autre: sur la même plaque il peut se produire des bourgeons nombreux. Cependant on doit supposer les conditions d'une mutation multiple dans une culture liquide plus restreintes que celles d'une culture sur milieu solide. C'est que dans celle-ci l'extension du mutant particulier est fortement restreinte, tandis que dans un verre à contenu liquide il peut vite se répandre sur tout le contenu. L'acidification causée par le premier mutant entravera probablement la tendance vers de nouvelles mutations, et même s'il en survient, la progéniture du premier mutant pourra prendre une forte avance, de sorte qu'elle vienne à constituer la partie majeure de la partie fermentaire de la culture. Dans les cas où la mutation ne survient que dans une petite fraction des verres il sera peu probable que deux individus bactériens du même verre muteront indépendamment l'un de l'autre; naturellement la possibilité n'en est pas tout à fait exclue.

Précédemment (vol. 17, p. 196) nous avons mentionné l'opinion divergente de *Burri* et d'autres. Ces dernières années il a été rapporté de différents côtés qu'une bactérie serait capable de transmettre ses pouvoir fermentatifs à d'autres bactéries, ou sous la condition que les deux espèces de bactéries se trouvent mélangées dans la même culture ou de manière qu'elles soient séparées par une membrane de collodion, à travers laquelle les produits du métabolisme peuvent diffuser. Par ce dispositif expérimental *Lisbonne*, *Nègre*, *Seigneurin* et *Roman* ont trouvé qu'un colibacille pouvait faire former de l'indol à un bac. aerogenes et lui faire perdre le pouvoir de former de l'acétyl-méthyl-carbinol, et que le bacille typhique pouvait faire former du H_2S au bac. paratyphi A et au colibacille. Il s'agit donc d'une analogie à la paragglutination induite. Nous espérons pouvoir plus tard contrôler ces expériences; nous examinerons ici s'il est possible d'observer des signes de phénomènes correspondants pour la fermentation mutative.

Une démonstration définitive de la justesse de nos opinions ordinaires sur la nature de la réaction mutative est à peine possible; mais on pourra examiner si les conséquences tirées de cette opinion et qui peuvent faire l'objet d'un contrôle, se confirment ou non. Pour de telles recherches notre collection de diverses formes mutatives de la même bactérie se trouverait bien appropriée. Supposons que nous avons un mélange de deux variantes (A et B) de la même bactérie dans un milieu renfermant une substance envers laquelle elles sont toutes deux à même de muter, si possible avec la même facilité, et qu'il existe un tel rapport entre la tendance mutative et la quantité de liquide que ce n'est qu'à titre exceptionnel qu'il surviendra plusieurs mutations indépendantes l'une de l'autre dans le même verre. Alors, selon l'opinion générale, après la survenue d'une mutation visible et macroscopique, tous les individus bactériens mutés doivent se rapporter ou à la variante A ou à la variante B tandis que la partie non mutée de la culture contient toujours les deux formes.

Afin de choisir des variantes bien appropriées, il fallait d'abord examiner la fréquence mutative des différentes cultures envers les différents sucres. La Du paraissant d'avance mais, comme on va voir, pas précisément à juste titre spécialement indiquée pour de pareilles expériences, on a commencé par essayer envers la Du toutes les formes, dont la formule était sans »Du«; les 6 qui étaient aussi sans »Ar« ont été essayées envers l'Ar, et les 8 qui outre la »Du« manquaient aussi le »Xy« (sans parenthèse) ont été essayées envers le Xy. Chaque culture a étéensemencée dans 6 verres de 2 c.c. e., qu'on a surveillés pendant 14 jours. Envers la dulcite les formes O, Xy, XyAr, Xy(Rh) et XyAr(Rh) ont muté; envers le Xy les formes O, Ar, (Rh) et Ar(Rh) ont muté dans tous les cas, de manière qu'au moins 4 verres jaunirent ou complètement ou partiellement dans le courant de 14 jours. Dans les autres cultures étudiées aucune acidification n'a été observée dans cette période.

Puisque dans aucun cas on ne vit de mutation envers l'Ar, on procéda à un examen renouvelé de toutes les 12 souches sans »Ar«, chacune dans 4 verres d'Ar de 8 c.c. Dans une période de 14 jours

10 des 12 cultures se teignirent en jaune dans un seul ou dans plusieurs verres. Des 10 souches tant la forme originaire que la forme mutée furent ensemencées sur plaques à Du, à Xy et à Rh. Dans tous les cas la forme originaire et la forme mutée se comportèrent de même au point de vue de la qualité; quant à l'intensité de l'acidification la forme *Xy(Rh)Ar* obtenue par l'expérience donna sur plaque de Xy une acidification plus rapide que celle donnée par la forme mère *Xy(Rh)*; cette différence fut confirmée par le réensemencement de respectivement 8 et 10 colonies des deux formes sur une nouvelle plaque à Xy. Donc nous tenons ici un exemple de ce que l'occurrence d'une mutation envers une espèce de sucre s'accompagne d'une augmentation du pouvoir fermentatif envers une autre espèce de sucre.

Nous voilà arrivés aux expériences principales. D'abord 12 verres d'ExB à 0.4 p. 100 de Du furent ensemencés d'un mélange à parties égales des variantes *XyAr* et *Xy(Rh)*; pour les soumettre à une comparaison on ensemença à part chacune de ces formes dans 6 verres.

Tous les verres étaient jaune verdâtre au bout de 5 jours, jaunes au bout de 6 jours.

6 jours après l'ensemencement on a disséminé de toutes les 24 verres sur une ou deux plaques à Du. Ainsi il s'est produit partout un mélange de colonies bleues et jaunes, bleues surtout. Les colonies bleues paraissaient toutes de grandeur normale (quelques colonies entravées ont pu nous échapper), et selon toute apparence elles appartenaient à la forme *Xy'*, celle-ci s'étant répandue pendant la culture en Du au dépens de la forme *Xy* originairement dominante.

De l'une des disséminations *XyAr* et de l'une des disséminations *Xy(Rh)* on a ensemencé 20 colonies bleues et 12 colonies jaunes sur plaque à Ar, à Xy et à Rh. Le résultat ressort de cet aperçu:

Réensemencement sur:				
	plaque à Ar	plaque à Xy	plaque à Rh	
de: <i>XyAr</i> , colonies bleues	jaune	bleu vert	entravé	
» colonies jaunes	jaune	jaune	entravé	
<i>Xy(Rh)</i> colonies bleues	bleu	bleu vert	non entravé	
» colonies jaunes	bleu	jaune	non entravé	

Les rapports à l'égard de Ar et de Rh ne sont donc pas influencés par les mutations (*Xy* \longrightarrow *Xy'* et *Xy* \longrightarrow *XyDu*) produites dans le milieu à Du.

En examinant 206 colonies bleues et 119 colonies jaunes provenant des plaques ensemencées de 9 des verres *XyAr* + *Xy(Rh)*, toutes les colonies bleues se comportaient comme »*XyAr* bleu«, et toutes les colonies jaunes comme »*XyAr* jaune«. Donc dans ces verres la forme *Xy(Rh)* a dû être si fortement refoulée qu'on n'a pu attraper que des colonies de la forme *XyAr*. Dans les cultures des plaques provenant des 3 verres restants (les nos. 1, 8 et 10) on put démontrer la forme *Xy(Rh)* aussi. On a fait ici un ensemencement supplémentaire

7 jours après l'ensemencement des verres; ce sont les chiffres d'ensemble pour les colonies provenant des deux ensemencements qu'on va donner ici. On a supprimé le résultat de l'examen fait envers le *Xy*, puisque, lors de l'ensemencement supplémentaire, il fut seulement ensemencé dans l'*Ar* et dans le *Rh*. Du premier ensemencement toutes les 92 colonies bleues étudiées se firent bleu verdâtre en *Xy*, tandis que les 60 colonies jaunes étudiées restèrent jaunes en *Xy*.

Plaques à Du	Réensemencement sur	
	plaque à <i>Ar</i>	plaque à <i>Rh</i>
du verre no. 1	196 colonies bleues	jaunes entravées
»	11 » bleues	bleues non entravées
»	67 » jaunes	jaunes entravées
»	1 » jaune	bleue non entravée
du verre no. 8	59 » bleues	jaunes entravées
»	4 » bleues	bleues non entravées
»	63 » jaunes	jaunes entravées
du verre no. 10	20 » bleues	jaunes entravées
»	11 » jaunes	jaunes entravées
»	1 » jaune	bleue non entravée

Dans le verre no. 8 36 des colonies étaient d'une couleur jaune beaucoup plus intense que celle des autres; mais, comme on vient de voir, ceci ne change rien. Le fait que parmi les colonies jaunes des deux verres on a trouvé et la forme *Ar* et la forme (*Rh*) (ou par la formule mutative complète: *XyDuAr* et *XyDu(Rh)*) doit être pris comme un témoignage de ce qu'il y avait dans les verres en question de la progéniture de deux mutants différents (au moins). Cependant ces mutations ont pu se développer indépendamment l'un de l'autre. C'est que l'époque égale à laquelle les 24 verres ont jauni, témoigne déjà d'une grande fréquence de mutation. Ceci est confirmé aussi par des expériences faites plus tard, dont il ressort que dans les verres à *Du* ensemencés avec la forme *O* on a trouvé de rares colonies jaunes déjà en ensemencant le premier jour, malgré que les verres ne commencèrent à jaunir qu'au bout de 14 jours.

Partie par cette raison, partie à cause du refoulement de la forme *Xy(Rh)*, cette expérience a manqué son but principal. Mais comme produit accessoire l'expérience globale sur les cultures des verres à *XyAr*, *Xy(Rh)* et *XyAr + Xy(Rh)* fournit tout de même sa contribution à l'éclaircissement de la constance des caractères acquis par la mutation, en tant que, parmi les 822 colonies examinées on a rencontré uniquement les deux combinaisons de caractères, auxquelles on pouvait s'attendre, à savoir: »*Ar*: jaune, *Rh*: entravé« et »*Ar*: bleu, *Rh*: non entravé«, tandis que les combinaisons »*Ar*: jaune, *Rh*: non entravé« et »*Ar*: bleu, *Rh*: entravé« n'étaient pas représentées.

Ensuite on a fait une seconde expérience où au lieu de la mutation de *Du* on a étudié la mutation d'*Ar*, qui paraît beaucoup plus sporadiquement. Pour cette expérience on a employé des mélanges des

formes (*Rh*) et *Du(Rh)*. Pour éviter qu'une forme refoulât l'autre on a fait d'abord, seulement à titre de renseignement, des expériences d'où il ressortit que les deux formes se faisaient le mieux équilibre, quand on les mélangeait dans la proportion de 1 à 3. Voici qu'on a ensemencé en tout 95 verres à Ar avec des mélanges des deux formes dans les proportions de 1 + 1, 1 + 2, 1 + 3 et 1 + 4, 23 ou 24 verres de chaque mélange. De l'ensemble de verres 7 en tout ont jauni au cours de 11 jours (ils appartenaient tous aux 3 mélanges derniers nommés). A cause de la rareté des mutations il fallait tenir pour peu probable la survenue de deux mutations indépendantes l'une de l'autre dans le même verre. L'expérience pouvait donc être considérée comme tout indiquée pour décider si une mutation d'une forme pouvait gagner l'autre. Des 7 verres on a fait un ensemencement lors du jour où ils jaunirent, dans la plupart des cas 1 ou 3 jours après; des colonies bleues et jaunes ainsi obtenues on a réensemencé sur une plaque à Du. Le tableau 12 en rend tout sommairement le résultat.

Tableau 12.
Couleur des colonies sur plaque à Ar:

	Bleue		Jaune ordin.		Intensément jaune	
	Du 0	Du +	Du 0	Du +	Du 0	Du +
2	3	19	0	219	0	0
6	76	8	0	171	0	0
6	19	66	0	98	0	0
8	52	1	0	101	0	0
9	0	88	30	0	2	1
9	57	0	72	0	0	0
11	2	37	44	0	0	0

La première colonne indique le nombre de jours s'écoulant jusqu'au jaunissement complet dans le tube à Ar.

Donc, pour 4 des verres les colonies mutées examinées appartenaient à la forme qui fermente la Du; pour 2 verres elles appartenaient toutes à la forme qui ne fermente pas la Du. Enfin 1 des 7 cultures se comportait d'une manière divergente en ce qu'on y trouva 3 mutants différents. Il est peu probable que ces 3 formes se soient produites indépendamment l'une de l'autre. On peut supposer que l'ordinaire mutant Ar jaune a continué sa mutation jusqu'à la forme intensément jaune, et que dans celle-ci une nouvelle mutation envers la Du s'est produite, mais d'autres explications sont aussi possibles. Pourtant il faut dire que l'expérience dans son entier soutient la supposition que toute la population mutée de bactéries dans un verre provient d'ordinaire d'un seul individu muté, si nous avons affaire à un assemblage de cultures, où la mutation est d'une occurrence si

rare que l'apparition de plusieurs mutations indépendantes l'une de l'autre dans de petits verres n'a pour elle qu'une mince vraisemblance statistique.

XII. Etudes sur les questions d'«entraînement» et de «co-entraînement».

De différents côtés il a été affirmé au cours des temps que, pour faire ressortir les réactions fermentatives caractéristiques, il fallait commencer par réensemencer une culture à plusieurs reprises dans un milieu riche en substances nutritives, additionné ou non de glucose. Ce point de vue a été mis en avant tout spécialement par *Neisser* qui prétendit qu'avant l'examen décisif des conditions fermentatives d'une souche de bactéries il fallait la réensemencer au moins 10 fois dans un bon milieu. Selon les expériences de *Courmont & Rochaix* une culture préparatoire dans un milieu renfermant un sucre fermentescible ne serait rien moins qu'appropriée à l'usage diagnostique, puisque de cette manière on ne faisait pas ressortir les réactions normales des bactéries, mais un pouvoir fermentatif exagéré:

«... un Bacille provenant d'une culture où il a produit une de ses fermentations normales, ensemencé dans un milieu renfermant un «sucre» non fermentescible normalement par lui, fait presque toujours fermenter ce dernier «sucre» par co-entraînement... Mais cette fermentation... est généralement temporaire et ne se reproduit pas à la génération suivante... Dans quelques cas, ces fermentations... se manifestent pendant plusieurs générations: le Bacille de Shiga ayant fait fermenté la glycose, produit la fermentation de la saccharose et de la maltose pendant 3 générations; le Bacille de Hiss, de la même origine, fermente l'inuline... et la sorbite...; le Bacille de Flexner ayant fermenté la dextrine et la maltose fermente à son tour le glycogène...; le Bacille de Flexner ayant fermenté la mannite fermente la sorbite et le glycogène...; le Bacille de Flexner ayant fermenté la glycérine fermente l'inosite et la sorbite... et le glycogène... le Bacille de Strong, provenant du milieu glyciné, produit, de même, la fermentation de l'inuline... et du glycogène... Une seule fois, nous avons obtenu une fermentation définitive par ce co-entraînement, celle de la galactose sous l'influence du Bacille de Shiga provenant d'un milieu glycosé».

Pour contrôler les résultats de *Courmont & Rochaix* nous nous sommes surtout tenus aux combinaisons indiquées dans le passage cité. 5 souches du bacille de Shiga et 5 souches du groupe de Flexner ont été ensemencées dans ExB. Le lendemain toutes les souches ont été réensemencées dans ExB et ExB à glucose, les souches du Bacille de Flexner en outre en ExB à mannite, ExB à maltose et ExB à glycérine (Dans les expériences dont nous parlerons dans ce chapitre les milieux à sucre ont renfermé 0,5 % de celui-ci, excepté le bouillon ordinaire à glucose, qui renfermait 1 %). Toutes les souches du bacille de Shiga ont fermenté le glucose en 1 jour, toutes les souches du bacille de Flexner aussi la mannite en 1 jour, et 3 d'entre elles le maltose

le 1^{er} jour; une des souches du bacille de Flexner a fait fermenter faiblement la glycérine, les autres n'ont pas fermenté la glycérine en 14 jours. Le lendemain on a réensemencé de toutes les cultures à fermentation et de tous les tubes à ExB, en glycérine, en sorbite, en glycogène et en inosite (une anse pour 2 c.c. e.). Toutes les souches de Shiga ont donné une acidification dans la glycérine au cours de plusieurs jours; une d'entre elles a fermenté aussi la sorbite en 1 jour; aucune d'entre elles n'a fermenté le glycogène ni l'inosite pendant 14 jours d'observation. Quant aux espaces de temps qui s'écoulaient jusqu'à l'acidification dans la glycérine la différence des cultures p. ex. celles d'ExB et celles d'ExB à glucose n'était pas importante, inclinant tantôt de l'un, tantôt de l'autre côté. Parmi les 3 souches de Flexner fermentant la maltose une a fermenté lentement la glycérine et le glycogène sans différence considérable entre les 4 séries quant à la vitesse; la sorbite et l'inosite ne furent pas fermentées. Les deux autres souches de Flexner ayant fermenté la maltose, produisirent la fermentation de la sorbite dans toutes les 4 séries en un jour, mais non pas celle de la glycérine, du glycogène ou de l'inosite. Parmi les deux souches de Flexner qui ne fermentaient pas le maltose, l'une fermenta dans toutes les 3 séries la sorbite en 1 jour, mais aucun des trois autres sucres; l'autre souche ne fermenta dans aucun cas aucun des 4 sucres.

Ces expériences montrent que c'était sans importance si une culture étaitensemencée d'un milieu exempt de sucre ou d'un milieu renfermant un sucre qu'elle venait de fermenter.

Les résultats de *Courmont & Rochaix* sont difficiles à expliquer. Il se peut que l'acidification qui s'est manifestée à la 1^{ère} génération soit due à du sucre entraîné, mais ceci n'explique probablement pas les cas où l'acidification a continué à des réensemencements réitérés.

Nous avons fait rentrer dans nos recherches une souche du bacille de Sonne isolée à l'Institut même, et possédant la propriété extraordinaire de fermenter la mannite d'une manière très inconstante. On l'aensemencé dans ExB et ExB à glucose: ce dernier milieu s'est acidifié en 1 jour. De ces deux verres on a continué d'ensemencer dans la mannite, laquelle,ensemencée d'ExB, ne s'est pas fermentée en 14 jours, tandis que dans le verre à manniteensemencé du verre à glucose il se produisit de l'acide le 2^e jour. En réensemencant dans un second verre à mannite l'acide se produisit le 1^{er} jour. Ici on aurait pu croire que la culture précédente en glucose eût éveillé un pouvoir latent de fermenter la mannite; mais une seconde expérience a montré que ce point de vue était insoutenable.

Cette nouvelle expérience entra dans un examen de l'importance des réensemencements de *Neisser*. On ensemença la souche du bacille de Sonne nommée ci-dessus, en ExB et de là dans de nouveau ExB, ExB + glucose, bouillon ordinaire et dans la gélose ordinaire. De chacun de ces milieux on a continué quotidiennement le réensemencement dans le même milieu; du 3^e verre (pour ExB: du 4^e) et du 10^e

verre (pour ExB: du 11^e) de chaque série on a ensemencé dans 3 verres à mannite. En voici le résultat: du verre ExB no. 4 un des verres à mannite jaunit le 2^e jour; de la culture de gélose no. 3, un des verres à mannite jaunit le 3^e jour. Tous les 22 autres verres à mannite restèrent bleus pendant 17 jours d'observation. Il s'agit vraisemblablement d'une fermentation mutative qui ne s'est manifestée que dans un petit nombre des verres et d'une manière fortuite; de l'expérience on ne peut conclure à aucune relation des milieux, employés pour la culture préparatoire, et la tendance fermentative.

Pour une seconde expérience on a appliqué 2 souches de Flexner F 5509/41—42 et F 15734/41—42, qui se distinguaient par des pouvoirs fermentatifs relativement faibles et en ce qu'elles ne produisaient pas d'indol. On est bien tenté de supposer qu'il s'agit d'un affaiblissement temporaire, qu'on pourrait supprimer à des réensemencements réitérés. Chacune des souches a été ensemencée dans ExB, ExB à glycose, le bouillon ordinaire, le bouillon ordinaire à glycose et sur la gélose ordinaire. De la 1^{re}, 3^e et 10^e culture de chacun de ces milieux on ensemença le contenu d'une anse dans la caséine digérée par la trypsine en 2 c.c. e. et dans les milieux que voici: Du, la sorbite, le Xy, le Rh, l'Ar, le maltose et le saccharose. Dans aucun cas une réaction d'indol nettement positive ne s'est manifestée. Dans les 4 milieux premiers nommés il n'y eut aucun cas de fermentation à 14 jours d'observation. Tous les verres à Ar jaunirent au cours de 1 ou de 2 jours, les verres à saccharose à F 5509 en 4 ou 5 jours, ceux à F 15734 ne jaunirent ou point du tout ou il se produisit dans 5 cas au bout de 12 ou 14 jours une coloration jaune partielle ou complète; dans un de ces derniers cas la fermentation tardive se produisit lors de l'ensemencement de la 3^e culture, dans les 4 autres cas lors de celui de la 10^e culture. Les verres à maltose présentèrent une variation un peu plus grande, la coloration jaune partielle ayant été relevée au bout de 7 à 8 jours, la coloration jaune complète au bout de 8 à 11 jours à ces exceptions près: F 5509 de la 1^{re} culture de gélose $+^5$ (c. à d. coloration jaune complète le 5^e jour), de la 3^e culture de gélose $+^1$, de la 10^e culture de gélose $(+)^2 +^3$ (c. à d. coloration jaune partielle le 2^e jour, complète le 3^e); F 15734 de la 10^e culture de glycose $+^3$ et de la 10^e culture de gélose $+^2$. En réensemencant dans un nouveau verre à maltose du verre de F 5509 positif au bout de 5 jours l'acidification se fit le 1^{er} jour; il paraît donc s'agir d'une fermentation mutative.

Pas des expériences supplémentaires nous avons vérifié que la fermentation de la mannite par les deux souches du bac. de Sonne et la fermentation du maltose par les deux souches du bac. de Flexner étaient bien de nature mutative.

Dans cette expérience il s'est montré dans quelques cas une tendance à une fermentation rapide après les 10 réensemencements; mais il ne paraît y avoir aucune raison de considérer ceci comme un témoignage de réactions fermentatives plus typiques que les réactions

primitives qui paraissent être restés invariables dès le 1^{er} réensemencement jusqu'au 3^e, en tout cas le »profit« en est si insignifiant qu'il ne justifie pas la grande consommation de milieu que nécessitent les réensemencements.

Les matériaux dont nous venons de faire l'étude sont très restreints, et on ne peut pas exclure la possibilité de l'existence de bactéries qui se comporteraient autrement; des expériences antérieures des réactions fermentatives des bactéries appartenant aux groupes *Salmonella* et dysentérie nous n'a pas donné l'impression que des réensemencements réitérés dans un milieu avec ou sans glycosé modifient considérablement les réactions fermentatives. Cependant nous avons toujours souligné l'importance d'un ensemencement d'une jeune culture préparatoire dans un milieu solide ou (mieux) liquide (bouillon ordinaire). Sans doute *Mikkelsen* dans son étude sur les colibacilles a-t-il trouvé dans beaucoup de cas une augmentation du pouvoir fermentatif après 3 réensemencements en bouillon à glucose; mais il se fondait sur une comparaison avec un ensemencement direct de cultures en piqûre; peut-être un seul ensemencement dans un bouillon ordinaire aurait-il eu le même effet que les 3 cultures dans du bouillon à glycosé.*)

On pourrait se demander si une bactérie mutant en présence de plusieurs substances envers lesquelles elle est capable de muter, muterait envers toutes à la fois; ce serait là un cas spécial de co-entraînement. Pour étudier cette question on ensemença la forme O de la souche typhique 6 dans 100 verres à ExB, contenant un mélange de Du et d'Ar, 0.4 % de chacun. Les premiers verres jaunirent au bout de 2, 3, 4, 4, 6, 6, 6, 8, 8, 8, 8, ... jours. De ces verres on fit une dissémination et le jour même du jaunissement et 2 jours après. Ainsi il y eut, dans tous les cas, et des colonies jaunes et des colonies bleues sur la plaque à Du; sur les plaques à Ar il y eut soit des colonies jaunes et bleues, soit seulement des colonies jaunes ou des colonies bleues. Un nombre de colonies bleues et jaunes sur les plaques à Ar et à Du provenant de 5 des cultures mutées furent réensemencées respectivement sur des plaques à Du et à Ar. Ainsi on était renseigné sur l'occurrence des 4 différentes combinaisons possibles d'une réaction positive et négative envers les deux sucres (tabl. 13).

Les chiffres 4 et 6 dans la première colonne indiquent l'époque du jaunissement du verre dont on avait ensemencé.

Dans toutes les 5 cultures il y avait des bactéries qui fermentaient la Du, mais non pas l'Ar. La mutation envers la Du n'est donc pas forcément accompagné d'une mutation envers l'Ar. Dans les cultures 4a et 6a—c il y avait des bactéries qui fermentaient l'Ar, mais non pas la Du. La mutation envers l'Ar n'est donc pas non plus forcément accompagné d'une mutation envers la Du. Quant aux 4 cultures

*) Voir aussi la prochaine communication.

Tableau 13.

Propriétés fermentatives des colonies:					{Ar	0	0	+	+
					Du	0	+	0	+
4a	colonies	sur	plaque	à	Ar	5	6	10	0
»	»	»	»	Du		10	11	7	0
4b	»	»	»	Ar		7	4	0	12
»	»	»	»	Du		13	13	0	1
6a	»	»	»	Ar		2	11	13	0
»	»	»	»	Du		4	13	21	0
6b	»	»	»	Ar		0	14	13	0
»	»	»	»	Du		2	12	12	0
6c	»	»	»	Ar		0	17	16	0
»	»	»	»	Du		4	15	20	0

Les chiffres 4 et 6 dans la 1^{re} colonne indiquent le nombre de jours s'écoulant jusqu'au jaunissement complet dans le tube à Ar + Du.

nommées on ne trouva (parmi le nombre restreint étudié) aucunes bactéries qui fermentaient les deux sucres. Par contre il y avait dans la culture 4b des bactéries fermentant et l'Ar et la Du; toutes celles qui fermentaient l'Ar se trouvaient parmi celles qui fermentaient la Du, mais la réciproque n'était pas vraie. Il paraît donc tout indiqué de supposer que la mutation envers l'Ar s'est produite dans la partie de la culture qui avait déjà muté envers la Du. Cette supposition est appuyé par le fait que déjà dans la dissémination de la culture mutant le 2^e et le 3^e jour on rencontra beaucoup de colonies fermentant la Du. Dans les 4 autres cas la mutation envers l'Ar doit s'être produite parmi les bactéries qui ne fermentent pas la Du. Dans aucun cas il y a donc des bactéries fermentant l'Ar et parmi les bactéries fermentant la Du et parmi celles qui ne fermentent pas la Du; pour chacune des 5 cultures, ou la dernière ou l'avant-dernière colonne est vide. Cette petite série d'expériences, en outre d'éclaircir le fait que la mutation d'un individu bactérien n'induit pas immédiatement une mutation envers une autre espèce de sucre, sert donc aussi à illustrer qu'un phénomène mutatif survenu dans une variante ne s'étend pas à une autre variante (chap. XI).

On aurait jugé bien utile de pouvoir employer au lieu de la mutation de Du très précoce, une mutation de la Du ou du Xy qui se développait d'une manière semblable à celle de la mutation d'Ar; peut-être ne serait-il pas impossible d'en trouver, mais en tout cas cela demanderait des préparations étendues.

Les expériences sur la »pureté« de la réaction mutative décrites dans ce chapitre et dans le chapitre précédent ne doivent pas être considérées comme de preuves décisives, mais plutôt comme un exemple de l'arrangement de pareilles expériences.

Résumé.

D'une souche typhique du type fermentatif 2 on a produit 24 variantes différentes et bien caractérisés, en la faisant muter envers une ou plusieurs des substances dulcité, l-arabinose, xylose et rhamnose dans les différentes combinaisons possibles; pour le xylose on comptait deux degrés de mutations.

Ces mutations se firent sans relation de dépendance. Dans un seul cas la mutation envers une espèce de sucre (l-arabinose) comporta une augmentation du pouvoir fermentatif, déjà acquis par mutation, envers une autre espèce de sucre (le xylose).

Les différentes formes mutatives envers le xylose se sont montrées inconstantes dans plusieurs cas. Voici les faits dont on a fait une étude détaillée: le mutant fermentant le xylose est entravé par la dulcité; cette entrave se laisse facilement surmonter par une nouvelle variation mutative; seulement par ce procédé le pouvoir de fermenter le xylose se perd. Ce pouvoir est recouvré, quand on fait muter la culture ou envers le xylose ou envers la dulcité.

Les bacilles typhiques du type fermentatif 1, c. à d. fermentant principalement le xylose, ne sont pas entravés par la dulcité. Il en est de même de quelques souches du type 2 mutées, mais la plupart de ces souches examinées se comportaient comme la souche premièrement étudiée, en ce quelles étaient entravées par la dulcité, et, quand cette entrave était supprimée, elles cessèrent de fermenter le xylose.

A deux occasions, on a observé la parte simultanée de la fermentation de la dulcité et du xylose.

La mutation du bacille typhique envers l'arabinose paraît avoir ceci de caractéristique que tant la fréquence de la mutation que le pouvoir fermentatif des mutants sont le plus grand dans les 2 ou 3 premiers jours; après, la fréquence de la mutation et, surtout, le pouvoir acidifiant des mutants décroissent.

A quelques souches différentes du groupe dysentérique on a partie fait subir une série de réensemencements dans des milieux différents, partie on les a cultivées dans des espèces de sucre différentes que principalement elles étaient à même de fermenter. Ces traitements préliminaires étaient d'ordinaire sans influence appréciable sur les autres pouvoirs fermentatifs des souches.

On a fait des expériences pour mettre en lumière les questions de savoir si la fermentation mutative en liquide nutritif était capable de s'étendre de la progéniture de l'individu premièrement mutée à d'autres parties de la culture, et si une bactérie mutant dans un mélange de deux espèces de sucre, envers lesquelles elle a le pouvoir de fermenter, mute envers les deux en même temps. On n'a observé aucuns signes certains de l'un ni de l'autre.

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WEITERE UNTERSUCHUNGEN ÜBER DIE COLIFLORA DES GESUNDEN MENSCHEN

Von *Beate Perch.*

(Eingegangen bei der Redaktion am 2. Oct. 1943).

In einer vorhergehenden Mitteilung von *F. Kauffmann* und *Beate Perch* wurde das Problem, ob der gesunde Mensch in seinen Faeces stets denselben Coli-Typ aufweise, oder ob ein häufiger Wechsel der Coliflora vorkomme, in Übereinstimmung mit verschiedenen Literaturangaben soweit gelöst, dass man zusammenfassend sagen konnte: »Wir haben es beim gesunden Menschen mit einer mannigfaltigen, stark wechselnden Coliflora zu tun und können oft in einer Faecesprobe gleichzeitig mehrere, verschiedene Coli-Typen nachweisen.«

Von den näheren Versuchsergebnissen dieser Arbeit sei kurz das folgende hervorgehoben: Die Faecesproben von 2 gesunden Menschen (K. und P.) wurden im Laufe von 5 Monaten je 14mal auf das Vorkommen verschiedener Coli-Typen untersucht.

Zur Differenzierung dieser Stämme wurde allein die Bestimmung der O-Antigene benutzt, während die H- und L-Antigene unberücksichtigt blieben.

Es wurden aus 14 Faecesproben der Versuchsperson K. 43 Coli-Kulturen, die zu 10 verschiedenen Typen gehörten, isoliert.

Aus der Versuchsperson P. wurden im Laufe von 5 Monaten aus 14 Faecesproben 41 Coli-Kulturen, die zu 22 Typen gehörten, gezüchtet.

Im Laufe des September wurden 4 Proben (= 10 Kolonien) entnommen, von denen die 2 letzten eine Reinkultur grampositiver Kokken enthielten. In den 3 übrigen Proben wurden 4 verschiedene Typen nachgewiesen, und zwar in jeder Probe ein neuer Typ.

Im October wurden gleichfalls 5 Proben (= 15 Kolonien) entnommen. Aus diesen 5 Proben wurden 13 verschiedene Typen isoliert, sodass jede Probe 2—3 verschiedene Typen enthielt.

Vom November bis zum Februar wurde pro Monat nur eine Probe untersucht, und zwar 4 Kolonien von jeder Probe. Die November-Probe gab 3 verschiedene Typen, unter welchen 1 Typ der vorigen Probe, P 10a, wieder isoliert wurde. Die December-Probe enthielt 2 neue Typen, in der Januar-Probe dagegen tauchte ein früher gefundener Typ, P 7b, wieder auf, wobei alle 4 untersuchten Kolonien zu diesem Typ gehörten. In der Anfang Februar untersuchten Probe wurde ein neuer Typ gefunden.

Es wurden also bei der Versuchsperson P. unter 41 Kulturen 22 verschiedene Typen gefunden, sodass die Mannigfaltigkeit der Typen hier viel grösser war als die der Versuchsperson K.

Diese Untersuchungen ergaben also, dass im Laufe von 5 Monaten in den Faeces der Versuchsperson K. 10 Typen und in denjenigen der Versuchsperson P. 22 Typen nachgewiesen wurden. Mit einer Ausnahme waren die O-Antigene der K- und P-Typen von einander verschieden.

Es blieb nun die Frage offen, ob diese mannigfaltige Coliflora konstant in ein und derselben Person anzutreffen sei, das heisst, ob alle diese Typen gleichzeitig und für lange Zeiträume im Darm anwesend seien, ohne dass es auf Grund der begrenzten Zahl (2—4) der untersuchten Kolonien möglich war, alle diese Typen stets gleichzeitig nachzuweisen. Es sollte daher geprüft werden, ob bei einer späteren Untersuchung (ca. 1 Jahr später) und dann bei Untersuchung zahlreicher Einzelkolonien derselben Probe wieder dieselben Typen gefunden wurden wie früher oder ob gänzlich neue Typen festgestellt wurden.

Ich habe deshalb vom 21. 11. 42 bis 20. 2. 43 in verschiedenen Zeiträumen 10mal meine eigenen Faeces erneut untersucht, und zwar 10—30 Kolonien jeder Probe mit allen 22 Immunsereen, die in der vorhergehenden Arbeit mit P bezeichnet waren, geprüft.

Bei der Isolierung der Kulturen ging ich folgendermassen vor:

Von der Ausstrichplatte (Drigalskiagar modifiziert) wurden von einzelnen Kolonien direkt eine Agarstich-Kultur (zur Aufbewahrung) und ein Bouillonröhrchen (zur Agglutination) angelegt. Sahen die einzelnen Kolonien alle gleich aus, so wurden wahllos 10 bis 30 Kolonien abgeimpft, sahen die Kolonien verschieden aus, so wurden auch diese abweichenden Kolonien berücksichtigt.

Das Bouillonröhrchen (ca. 20 ccm) wurde nach ca. 20 std. Bebrütung bei 37° C. 1 Std. lang auf 100° C. erhitzt und zur Konservierung mit 1 ccm einer 5 % Formalinlösung versetzt. Die gekochten Bouillonkulturen wurden dann mit den 22 P-Immunsereen, ab Verdünnung 1:20, agglutiniert. Es wurden zuerst 2 Röhrchen (1:20 und 1:40) pro Stamm benutzt; kam es in beiden Röhrchen zur Agglutination, so wurden die Stämme austitriert, d. h. bis zur Verdünnung 1:5120 angesetzt. Die Agglutination wurde nach ca. 20 std. Aufenthalt im Wasserbade von 50° C. abgelesen.

Über die sonstige angewandte Technik ist bereits in der vorhergehenden Mitteilung von F. Kauffmann und Beate Perch ausführlich berichtet worden, sodass hierauf verwiesen werden kann.

Ergebnisse der Untersuchungen.

Die einzelnen Faecesproben wurden mit römischen Zahlen, die einzelnen Kulturen innerhalb der Proben mit arabischen Zahlen versehen, sodass z. B. die erste Kultur der ersten Probe die Bezeichnung P I₁ bekam.

Falls eines der benutzten 22 Seren, die früher hergestellt waren, einen der frisch isolierten Stämme bis zum Titer agglutinierte, so

Tabelle 1.

Faeces-probe	Datum der Entnahme	Zahl der untersuchten Kolonien	Zahl der gefundenen Serotypen	Nahe O-Beziehungen zu früheren P-Typen	O-Identität mit früheren P-Typen	Kulturelle Identität mit den zugehörigen P-Typen
P I	25.11.42	20	P I ₁ (8×) P I ₁₀ (1×) X-Gruppe (11×)	P I ₁ - P 10b	P I ₁₀ = P 12b	—
P II	28.11.42	18	P I ₁ (8×) P II ₅ (2×) X-Gruppe (8×)	P I ₁ - P 10b P II ₅ - P 2a		— —
P III	6.12.42	30	P I ₁ (28×) X-Gruppe (2×)	P I ₁ - P 10b		—
P IV	4.1.42	18	P I ₁ (3×) P IV ₂ (13×) X-Gruppe (2×)	P I ₁ - P 10b	P IV ₂ = P 7b	+
P V	5.1.43	15	P IV ₂ (14×) X-Gruppe (1×)		P IV ₂ = P 7b	+
P VI	26.1.43	15	P IV ₂ (13×) U 9 (2×)		P IV ₂ = P 7b	+
P VII	31.1.43	15	P IV ₂ (6×) U 9 (9×)		P IV ₂ = P 7b	+
P VIII	6.2.43	25	P IV ₂ (1×) P VIII ₈ (18×) U 9 (6×)		P IV ₂ = P 7b P VIII ₈ = P 9c	+ +
P IX	13.2.43	10	P I ₁₀ (2×) P IX ₇ (2×) U 9 (1×) X-Gruppe (5×)		P I ₁₀ = P 12b P IX ₇ = P 7d	— +?
P X	20.2.43	30	U 9 (28×) X-Gruppe (2×)			
10 Proben	25.11.42— 20.2.43	196		2 Typen	4 Typen	2 Typen

Zeichenerklärung:

Die eingeklammerten Zahlen geben die Häufigkeit der Befunde an.
 X-Gruppe = Stämme die keine serologische Beziehung zu den früheren P-Typen (= aus der Versuchsperson P. isoliert) besaßen.

U 9 = einer der häufigsten, pathogenen Typen nach *F. Kauffmann* (Typ 2 A).

+ = kulturell identisch.

— = kulturell nicht identisch.

+ ? = kulturelle Identität zweifelhaft.

wurde das kulturelle Verhalten dieses Stammes genau untersucht. Ausserdem wurde festgestellt, ob der betreffende Stamm (auf 100° C. erhitzte Kultur) das betreffende O-Serum völlig erschöpfte. War dieses der Fall, so wurde mit dem Stamme ein O-Serum hergestellt und, falls er O-inagglutinabel war, ausserdem noch ein L-Serum (mit lebenden Bakterien). Ferner wurde ein H-Immunserum gewonnen, wenn der Stamm beweglich war (mit 5 std. Formalin-Bouillonkultur).

Aus der Tabelle 1 ist zu ersehen, wann die einzelnen Faecesproben entnommen wurden, wieviele Kolonien untersucht sind und wieviele Stämme mit den früher isolierten P-Stämmen ganz oder teilweise übereinstimmten.

Aus der Tabelle 1 geht hervor, dass im Laufe von 5 Monaten 196 Kulturen (von je einer Einzelkolonie aus angelegt) untersucht wurden. Unter diesen 196 Stämmen befanden sich 70, die zu 4 früher nachgewiesenen Typen gehörten, d. h. dass sie dasselbe O-Antigen wie diese Typen hatten. Es handelt sich um die Typen P 7b, P 7d, P 9c und P 12b.

Der Typ P 7b = P IV₂ wurde in 4 Faecesproben (P IV — P VII) gefunden und zwar in 3 dieser Proben weit vorherrschend (von 63 Kulturen 46mal).

Der Typ P 7d = P IX₇ wurde nur in einer Faecesprobe 2mal unter 10 Kulturen nachgewiesen. Der Typ P 9c = P VIII₈ wurde ebenfalls nur in einer Probe unter 30 Kulturen 18mal gefunden.

Der Typ P 12b = P I₁₀ wurde aus der ersten Probe unter 20 Kulturen 1mal und aus der neunten Probe unter 10 Kulturen 2mal nachgewiesen.

Von diesen 4 Typen waren nur 2: P IV₂ und P VIII₈ mit den entsprechenden Typen P 7b resp. P 9c kulturell identisch, während die beiden anderen P I₁₀ und P IX₇ kulturell verschieden waren.

In der folgenden Tabelle 2 sind diese Unterschiede angegeben.

Während bei den Typen P I₁₀ und P 12b sichere kulturelle Unterschiede bestehen, sodass es sich um 2 verschiedene Vergärungstypen

Tabelle 2.

	P I ₁₀	P 12b
Adonit	+ 3-6	— 30
Dulcit	— 30	+ 1
Salicin	+ 2-3	— 30
Indol	+	—
	P IX ₇	P 7d
Rhamnose	+ 2-3	+ 3-6
Indol	+	—

handelt, sind die beiden Typen P IX₇ und P 7d vergärungsmässig fast gleich, unterscheiden sich aber hinsichtlich ihrer Indolbildung.

Falls aber innerhalb desselben Stammes indolpositive und indolnegative Varianten auftreten, so ist dieser Differenz keine Bedeutung beizulegen, sodass diese beiden Kulturen als zusammengehörig betrachtet werden könnten.

Es sei bei dieser Gelegenheit noch erwähnt, dass nähere Untersuchungen über die Saccharose-Vergärung von Coli-Stämmen, die Unregelmässigkeit dieser Vergärung bei verschiedenen Stämmen ergaben.

Mit Hilfe serologischer Methoden liess sich bei diesen 4 Typen oder vielmehr Paaren von Typen:

P 7b ——— P IV₂
 P 7d ——— P IX₇
 P 9c ——— P VIII₈
 P 12b ——— P I₁₀

die völlige Identität der O-Antigene nachweisen, und zwar auf Grund gekreuzter Absorptionsversuche.

Bei den 3 ersten Typen-Paaren konnten L-Antigene nicht mit Sicherheit nachgewiesen werden, sodass die Frage offen bleiben muss, ob sämtliche Körperantigene dieser Kulturen identisch sind oder nicht. Die letzten Typen P 12b und P I₁₀ besaßen L-Antigene, die sich aber serologisch von einander unterschieden, obwohl sie gemeinsame Partialantigene enthielten. Aus diesem Grunde müssen wir diese beiden Kulturen, die sich auch kulturell von einander unterscheiden, als 2 verschiedene Typen betrachten.

H-Antigene waren nur bei den beiden Stämmen P 9c und P VIII₈ nachweisbar und erwiesen sich serologisch als identisch, sodass wir berechtigt sind, diese beiden Kulturen als gleich zu betrachten, zumal sie auch in kultureller Hinsicht übereinstimmen.

Auf Grund dieser Ergebnisse können wir feststellen, dass im Laufe der jetzigen Untersuchung mindestens 2 (eventuell 3) der früher nachgewiesenen 22 P-Typen wiedergefunden wurden. Während also von 196 Kulturen 70 dasselbe O-Antigen wie 4 frühere Typen besaßen, bestanden bei 49 weiteren Kulturen starke O-Antigenbeziehungen zu 2 früheren Typen.

P I₁ ———> P 10b
 P II₅ ———> P 2a.

Da diese beiden Typen aber nicht dasselbe O-Antigen enthielten und ausserdem kulturell verschieden waren, müssen sie als je 2 besondere Typen betrachtet werden.

In der Tabelle 3 sind die O-Antigen-Unterschiede zwischen diesen Kulturen zusammengestellt.

Bei der Untersuchung dieser zuletzt genannten Stämme P 2a und P II₅ wurde eine Beobachtung gemacht, die näher erörtert werden soll.

Tabelle 3.
O-Immunseren.

agglutiniert mit 100%-Kultur	P 10b		P I ₁	
	nicht absorb.	absorb. mit P I ₁	nicht absorb.	absorb. mit P 10b
P 10b	5120	0	5120	0
P I ₁	5120	0	5120	320
	P 2a		P II ₅	
	nicht absorb.	absorb. mit P II ₅	nicht absorb.	absorb. mit P 2a
P 2a	5120	0	5120	0
P II ₅	5120	0	5120	5120

Während das P 2a-Serum beide Kulturen P 2a und P II₅ stark agglutinierte, hat das zuerst hergestellte O-Serum des Stammes P II₅ nur die homologe Kultur stark bis 1:5120 agglutiniert, während es die P 2a-Kultur nur schwach bis 1:20 \pm flockte. Ich stellte daher 2 weitere O-Seren mit dem Stamm P II₅ her und erhielt so Immunseren, welche beide Kulturen hoch agglutinierten, das eine agglutinierte die Kultur P 2a bis 1:1280 ++, 1:5120 \pm , während das andere diese Kultur bis 1:5120 ++ agglutinierte. Der homologe Stamm wurde von beiden Seren bis zur Verdünnung von 1:5120 geflockt. Dieser Versuch zeigt, dass das zuerst benutzte Kaninchen nicht imstande war, Antikörper gegen das übergreifende Partialantigen dieses Stammes zu bilden. Es empfiehlt sich daher, stets mehrere Kaninchen (mindestens 2) zur Serumherstellung zu benutzen und ihre Seren zu mischen, um auf diese Weise individuelle Unterschiede auszuschalten.

Ausserdem habe ich mit dem Stamme P II₅ 3 Immunseren mit lebenden Bakterien hergestellt, die alle beide Stämme P II₅ und P 2a hoch agglutinierten.

Die bereits erwähnten Absorptionsversuche haben jedoch gezeigt, dass diese Kulturen, trotz naher O-Antigenbeziehungen, nicht zu dem gleichen Typ gehören, sodass wir also weder den Typ P 2a noch den Typ P 10b wiedergefunden haben.

Die restlichen 77 Kulturen hatten keine O-Antigenbeziehungen zu den 22 früher festgestellten P-Typen und sind in der Tabelle 1 mit X-Gruppe resp. U 9 bezeichnet, da ihre serologischen Beziehungen unter einander nicht näher untersucht wurden (betreffs des U 9-Stammes siehe weiter unten). Wir können also nicht wissen, aus wievielen Typen diese Gruppe besteht.

Um zu sehen, ob einige der untersuchten Kulturen, die nicht mit den bekannten P-Typen identisch waren, eventuell zu den von F. Kauffmann aufgestellten ersten 20 Coli-Gruppen (resp. Typen) gehörten, habe ich diese Kulturen mit allen 20 Seren geprüft. In diesen

20 Gruppen (resp. Typen) sind die am häufigsten gefundenen, pathogenen Coli-Typen, die aus Fällen von Cystitis, Pyelitis, Cholecystitis und Peritonitis isoliert waren, enthalten. Über die Beziehungen der bereits bekannten P-Typen zu den erwähnten 20 Gruppen hat bereits *F. Kauffmann* berichtet.

Wie die Tabelle 1 zeigt, ergab die Untersuchung, dass in 5 Proben (= 95 Kolonien) einer der häufigsten pathogenen Stämme, der Stamm U 9 (Typ 2 A), 46mal nachgewiesen wurde. Um die Identität dieser Kultur mit dem U 9-Stamme zu klären, wurde von einer dieser Kulturen, P X₁, ein O-Serum hergestellt sowie das kulturelle Verhalten untersucht. Der Absorptionsversuch ergab, dass beide Stämme, die kulturell identisch waren, dasselbe O-Antigen besaßen. Ferner enthielt der Stamm P X₁ wahrscheinlich dasselbe L-Antigen (L 1) wie der Stamm U 9, da er imstande war, ein L-Serum des Stammes U 9 reslos zu erschöpfen. Er wurde von L 1-Serum bis zum Titer agglutiniert.

Es sei nebenbei erwähnt, dass ich das L 1-Antigen noch bei 2 weiteren Stämme, P IV₂ und P I₁ nachweisen konnte.

Besprechung der Ergebnisse.

In einer früheren Untersuchung über die Coliflora von 2 gesunden Personen fanden *F. Kauffmann* und *Beate Perch*, dass es sich beim gesunden Menschen um eine mannigfaltige, wechselnde Coliflora handelt. Wie weit aber diese Mannigfaltigkeit ging, und ob eine gewisse Konstanz innerhalb dieser Mannigfaltigkeit bei ein und derselben Person bestand, d. h. ob alle gefundenen Typen stets gleichzeitig zur Stelle waren, konnte in diesen Untersuchungen auf Grund der begrenzten Zahl der Kolonien (2—4) und der begrenzten Zeit nicht geklärt werden. Daher wurden 1 Jahr später erneute Untersuchungen der Faecesproben von einer der beiden Versuchspersonen (P.) vorgenommen, wobei von jeder Probe 10—30 Kolonien geprüft wurden. Es sollte untersucht werden, ob man bei diesen späteren Untersuchungen dieselben Coli-Typen (P), die vor 1 Jahre aufgestellt waren, wiederfinden konnte oder nicht.

Die Feststellung dieser Tatsache stösst nun aber auf grosse, oft unüberwindliche Schwierigkeiten. Um die Identität von 2 Coli-Kulturen zu sichern, muss nämlich nachgewiesen werden, dass sie dieselben O-, L- und H-Antigene besitzen, und dass sie ausserdem in kultureller und vergärungsmässiger Hinsicht völlig identisch sind. In Wirklichkeit liegen die Verhältnisse innerhalb der Coli-Gruppe aber so, dass wir in der Mehrzahl der Fälle serologisch nur die Identität der O-Antigene feststellen können. Die L-Antigene sind nach *F. Kauffmann* speciell bei Faeces-Stämmen nur in einem Teil der Fälle nachweisbar, ebenso die H-Antigene, sodass also in der Regel nichts ande-

res übrig bleibt, als die Identität oder Verschiedenheit der O-Antigene und das kulturelle Verhalten zu berücksichtigen. Stimmen 2 Stämme in dieser Hinsicht unter einander überein, so kann man sie mit einer grossen Wahrscheinlichkeit als identisch bezeichnen, wenn auch diese Schlussfolgerung nicht absolut sicher ist. Andererseits kann darüber kein Zweifel bestehen, dass 2 Stämme von einander verschieden sind, wenn ihre O-Antigene verschieden gebaut sind, und wenn sie deutlich verschiedenen Vergärungstypen angehören.

Unter Berücksichtigung dieser Merkmale ergaben die vorliegenden Untersuchungen, dass 2 frühere Typen, P 7b und P 9c, wiedergefunden wurden; jedenfalls stimmten die frisch isolierten Kulturen in kultureller Hinsicht und in ihren O-Antigenen mit diesen Typen überein. Möglicherweise wurde noch ein dritter Typ, P 7d, wiedergefunden, doch stimmten diese Stämme in kultureller Hinsicht nicht völlig überein. Lassen wir diesen fraglichen Fall ausser Betracht, so wurden von den 22 früher aufgestellten P-Typen 2 dieser Typen 1 Jahr später wiedergefunden. Von diesen war der eine, P 7b, in 5 Faecesproben, die im Laufe eines Monats untersucht wurden, vorherrschend.

Dieses Ergebnis zeigt, dass die Faecesflora des gesunden Menschen mannigfaltig und wechselnd ist, dass aber daneben bestimmte Typen nach einem Jahre wieder aus demselben Menschen isoliert werden können. Ein völlig klares Bild der Coliflora lässt sich aber noch nicht gewinnen, da hierzu weit ausgedehntere Untersuchungen an mehreren Personen über viele Jahre hinaus in Zwischenräumen von 1—2 Monaten erforderlich wären, über die wir heute nicht verfügen. Bei der verschiedenen Verteilung der in *einer* Faecesprobe anwesenden Typen müsste man ca. 100 Kolonien einer jeden Probe genau analysieren — eine Arbeit, die praktisch undurchführbar ist.

Von den hier erhobenen Befunden sei abschliessend noch auf die wiederholte Feststellung des U 9-Stammes (Typ 2 A nach *F. Kauffmann*) hingewiesen, da es sich hierbei um einen der häufigsten »pathogenen« Coli-Typen handelt. Wie schon früher von *F. Kauffmann* betont, kommen derartige Typen auch in den Faeces gesunder Menschen vor, sodass nur der Prozentsatz ihrer Häufigkeit im normalen und pathologischen Material verschieden ist.

Ich bin Herrn Dr. *F. Kauffmann* für seine Hilfe bei der Abfassung dieser Publikation zu grossem Dank verpflichtet.

Zusammenfassung.

Bei der Untersuchung von 10 Faecesproben (196 Coli-Kulturen) einer Versuchsperson P., deren Coliflora vor einem Jahre bestimmt

wurde, konnten von 22 früher festgestellten Coli-Typen mindestens 2 (eventuell 3) dieser Typen wiedergefunden werden.

Trotzdem man es also im allgemeinen mit einer mannigfaltigen, wechselnden Coliflora zu tun hat, so können doch einige Typen lange Zeit hindurch nachweisbar sein.

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CULTIVATION OF BACTERIA FROM THE BLOOD, ESPECIALLY WITH EMPLOYMENT OF CITRATE VENULE*)

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(Received for publication Oct. 28th, 1943).

The introduction of venules in the blood culture technique has simplified this method of examination, and it has contributed increasingly to make blood cultures a routine method of clinical examination. The earlier in the course of a disease a bacterium is cultivated from the blood of the patient, the greater is the probability that the respective bacterium be the cause of the disease; and this probability practically becomes a certainty when the same microbe is obtained repeatedly in cultures from the patient's blood.

In some cases, however, the microbe grown from the blood cannot be reckoned as the primary cause of the disease but has to be looked upon as a secondary invader of the macroorganism favored by a lowered resistance of the host. In every case of infection — *i. e.*, invasion of the internal functional field of the macroorganism by a microorganism — the course of the infection will depend on two things: the resistance of the macroorganism and the virulence of the microorganism. According to Butler (2), secondary invasion by bacteria which have nothing to do with the primary infection is not uncommon in acute infectious diseases involving the upper respiratory passages — *e. g.*, scarlet fever, measles, diphtheria, and influenza. Secondary infection is encountered also in other diseases which greatly lower the resistance of the macroorganism — *e. g.*, agranulocytosis, cancer, and typhoid fever. These invaders are not only bacteria of unquestionable pathogenicity — as, for instance, hemolytic streptococci — but we have to reckon with the fact that also bacteria of

*) A technical contrivance, manufactured by Bayer, for the withdrawal of blood and other body fluids. It is always sterile and ready for use. It works automatically by a vacuum in the receiving tube.

dubious pathogenicity — *e. g.*, diphtheroid bacteria, staphylococcus albus, and *B. faecalis alkaligenes* — under these conditions now and then are able to assert themselves as pathogenic microbes. As these bacteria often are due to contamination of the cultures, being admixed to the blood from the skin during the withdrawal of the blood or in the handling of the blood in the laboratory, it is obvious that the significance of a positive cultural result often may be doubtful.

If, for instance, growth of typhoid bacilli or meningococci is obtained from the blood, there can be no doubt about the diagnosis. On the other hand, if non-hemolytic streptococci are demonstrated, the interpretation of the result is not so easy, even though non-hemolytic streptococci very often are isolated from patients with subacute endocarditis. In this disease, as a rule, there is a very massive invasion of the blood stream, so that quantitative and frequent blood cultures are of particular importance in such cases (Butler (2)). Generally, diphtheroid bacteria obtained from the blood are seldom reckoned to have brought about the disease of the patient (Thompson (11)). Nor do most investigators attach any particular significance to staphylococcus albus as a cause of disease, whereas generalized infection with non-pigment-producing staphylococci has been reported by several authors.

To the prognosis, the bacteriological findings in cultures from the blood are of great importance. As a rule, generalized infection with colon bacilli is of a mild character — except in infants, in whom it is very dangerous (Penfold & Butler (11)). In subacute endocarditis caused by non-hemolytic streptococci the symptoms often are not alarming, but the prognosis is bad. Also growth of hemolytic streptococci in blood cultures gives most often a poor prognosis.

The frequency of positive results from the blood cultures will depend both on the nature of the infection and on the method of cultivation. The amount of blood employed, the choice of culture medium, the treatment of the blood before its addition to the medium, the incubation period, the methods for observation of possible growth and the measures taken to avoid contamination vary with the different investigators. The aim is to provide the best cultural conditions possible for the bacteria which may be present in the blood and to abolish or reduce the bactericidal effect of the blood on these bacteria as far as possible. Many authors state it is advisable to make the blood cultures at bedside instead of waiting till the blood reaches the laboratory. In many cases, this will make it unnecessary to add any anticoagulant to the blood. Further, the effect of such anticoagulants on delicate bacteria is partly unknown. Besides, in this way, the bactericidal properties of the blood will have less time to assert themselves.

In the withdrawal of the blood a careful disinfection of the skin is highly important; and after its disinfection, the skin must not be

touched by the finger at the site where the needle is to be introduced into the vein, which may be marked out before the disinfection.

The simplest method for a reduction in the bactericidal effect of the blood is dilution with the culture medium employed, often broth. The degree of dilution varies with the various investigators, from 1:5 to 1:30. Addition of sodium citrate diminishes the bactericidal properties of the blood, but it may also have an inhibitory effect on the growth of certain pathogenic bacteria (Brown (1), Wright (13)). Another method for reduction in the bactericidal effect of the blood is addition of numerous tiny particles to the culture medium (Cummins (5), Kracke & Teasley (7)). Also bile has this effect. Some investigators recommend addition of sterile active trypsin to the culture medium or to the blood for lowering of its bactericidal power (Douglas & Colebrook (6), Colebrook (3), Colebrook and Hare (4), Penfold & Butler (9), and Butler (2)). Also coagulation of the blood is said to be prevented in this way. According to Massa & Battistini (8) a similar effect is obtained by addition of sodium polyanetholsulfate, which is placed on the market under the designation »liquoid«.

In some infectious diseases — *e. g.*, undulant fever — particular cultural methods are required.

Naturally it is difficult to give any numerical frequency of the positive results which may reasonably be expected in cultivation from the blood — for one thing, of course, because the outcome will largely depend on the patient material. According to Butler (2), most examiners employing ample amounts of broth obtain but 10 % positive results, at the most, among the patients in general hospital wards, whose clinical symptoms constitute indications for the employment of blood cultures. Butler (2), employing broth and bile, obtained 10—15 % positive results; but when she employed some additional culture media — including an anaerobic medium with an addition of active trypsin — she was able to increase the incidence of positive results to 25—30 %. The anaerobic medium mentioned is claimed to be particularly suitable for the cultivation of anaerobic non-hemolytic streptococci — for instance, in puerperal sepsis or after abortion.

Writer's Studies.

The cultural method employed in recent years in the Diagnostic Department of the State Serum Institute as the general routine method was introduced here by Vesterdal Jørgensen (12), who has given a detailed account of it. In addition, particular culture media and methods are employed for special purposes — *e. g.*, cultivation from the blood of *Brucella abortus* Bang and typhoid bacilli. In the following, only the general routine method will be mentioned.

The blood which is to be examined by cultivation is sent to the

institute in a venule with a capacity of 8 cc. and containing 1.5 cc. of 3.8 % sodium citrate solution. After introduction of the needle into one of the cubital veins of the patient, the venule is filled with blood through a manipulation of the venule so as to open its valve. Owing to the underpressure in the tube, the blood is aspirated into the venule, which then is shaken a little — for mixing of the blood and citrate. Thus clotting of the blood is prevented.

When the venule arrives at the institute, its rubber stopper is removed, and by means of Pasteur pipettes the blood is distributed in test tubes containing 5 culture media: serum broth, Truche's medium, semifluid agar, cysteine agar, and cysteine broth covered by a layer of mineral oil. The last two media are anaerobic. The distribution of the blood in the media is carried out so that 4 tubes of each medium are inoculated with the blood, making a total of 20 tubes for each specimen of blood. The transfer of the blood is performed by means of 4 Pasteur pipettes, 5 tubes with different media for each pipette. In the last couple of years, one of the anaerobic media — cysteine agar — has been omitted, reducing the total number of tubes for each specimen to 16. The rack holding the 16 tubes is incubated at 37°, and the cultures are under daily observation for one week. In the first years the cultures were inspected daily for two weeks, but experience showed that the results were just as good with one week's incubation as with two weeks', the shorter incubation period was adopted.

The purpose of this cultural method is to offer the bacteria possibly present in the blood some differing conditions for growth, including different oxygen tension, and to make it practicable to estimate the degree of the bacteremia, as the eventual growth in the tubes with some training as a rule may be observed directly with the naked eye.

The culture media here employed are prepared as follows:

1. *Serum broth*: To 1 liter of sterile beef broth, 50 cc. of sterile ox serum is added; the mixture is filtered through a sterile Berkefeld filter at low underpressure.

2. *Truche's medium*: 1 liter tap water, 40 g. of peptone Chapoteaut, 2 g. glucose, and 2 g. sodium chloride. The mixture is boiled, alkalized to $p_{H}=7.8$, filtered and steam-sterilized at 100° for 20 min. on 3 successive days.

3. *Semifluid agar*: 1 liter of sterile beef broth with $p_{H}=7.4$ is heated on water-bath to 50°. To this is added 85 cc. of melted sterile 2.5 % water agar. After tubing of the mixture, steam sterilization at 100° for 10 min.

4. *Cysteine agar*: 1 liter of sterile beef broth is heated on water-bath. Then 170 cc. of melted sterile 2.5 % water agar is added, together with 3 g. cysteine hydrochloride (weighed off sterily). p_{H} is adjusted to 7.2, and 10 g. glucose is added. After tubing of the mixture, steam sterilization at 100° for 10 min.; then rapid cooling.

5. *Cysteine broth*: Same amounts of cysteinehydrochloride and glucose

Table 1.

Survey of the Bacteria Grown in the Blood Cultures.

grown from 504 (5.7%) specimens from 464 patients				
Staphylococcus albus ¹	»	416 (4.7%)	»	213 »
Non-hemolytic strept. ^{11, 12}	»	160 (1.8%)	»	144 »
Staph. aureus ²	»	66 (0.8%)	»	65 »
Coryneiform bacteria ⁶	»	63	»	59 »
Hemolytic strept. ³	»	60	»	57 »
Pneumococci ^{8, 14}	»	29	»	26 »
Bac. typhi ⁴	»	29	»	24 »
Bacteroids ^{9, 13}	»	16	»	15 »
Colon-like bacteria ⁷	»	15	»	13 »
Bac. coli ⁵	»	10	»	10 »
Sarcina	»	9	»	9 »
Staph. citreus	»	8	»	8 »
Meningococci	»	5	»	5 »
Bac. paratyphi	»	5	»	5 »
» subtilis	»	4	»	4 »
» proteus	»	4	»	2 »
Gram-neg. coccobact. ¹⁰	»	3	»	3 »
Bac. pyocyaneus	»	2	»	2 »
» faecalis alkal.	»	1	»	1 »
» influenzae Pfeifferi	»	1	»	1 »
» anthracis	»	1	»	1 »
» phlegm. emphysem.	»	1	»	1 »
Br. abortus Bang	»	1	»	1 »
Salmonella Dublin	»	1	»	1 »
» Oranienburg	»	1	»	1 »
Gram-neg. cocci in long chains	»	1	»	1 »
Total		1415	»	1135 »
Specimens with combined bact inf.		152	»	77 »
Total No. of positive specimens		1567 (17.8%)	»	1212 »

- 1) 2 times from 32 patients; 3 times from 2 pts.; 5 times from 1 pt.
 2) 2 » » 16 »
 3) 2 » » 4 »
 4) 2 » » 3 »
 5) 2 » » 2 »
 6) 2 » » 1 »
 7) 2 » » 1 »
 8) 2 » » 1 » 3 times from 1 pt.
 9) 2 » » 3 » 3 » » 1 »
 10) 3 » » 1 »
 11) 2 » » 36 » 3 » » 23 » 4 times from 9 pts.
 5 » » 5 » 6 » » 8 » 7 » » 2 »
 10 » » 1 » 14 » » 1 »
 12) In 1 case growth only under anaerobic conditions.
 13) As far as the writer knows, this group is not reported before in this country.
 14) The distribution of the pneumococcus types on the patients was:
- | | | |
|----------------------|---------------------|---------------------|
| Type 1: 32 patients. | Type 6: 2 patients. | Type 12: 1 patient. |
| » 7: 6 » | » 8: 2 » | » 19: 1 » |
| » 3: 4 » | » 18: 2 » | » 22: 1 » |
| » 10: 3 » | » 2: 1 » | » 23: 1 » |
| | | » 32: 1 » |

as in the preceding, but no agar. A layer of mineral oil, 1—1½ cm. in height, is placed in each tube.

In the past 5 years the number of citrate venules with blood for cultivation has increased to five times the initial number, showing a steadily increasing interest in this form of examination. Altogether 8799 venules were received in this period. Of this total, 1567 (*i. e.*, 17.8 %) gave a positive result of the cultivation. Altogether 24 different kinds of bacteria were isolated; they are recorded in Table 1.

Undoubtedly some of the bacterial groups entered in Table 1 have included several near-related bacterial species. The »positive« venules came from 1212 patients, as growth often was obtained on repetition of cultivation from the same patient. The results are shown in Table 1. In 152 specimens of blood from 77 patients the bacterial findings were combined as follows: 1) more than one bacterial species were isolated from the venule, or 2) repeated cultivation from the same patient yielded two or more different kinds of bacteria (Table 2). In several cases, presumably, this outcome was due to contamination; but it is also possible that there may have been a secondary infection on the basis of lowered resistance.

The contaminations, as mentioned, may come from the skin at the withdrawal of the blood; but it is also possible that they may take place in the laboratory on opening of the venule and distribution of the blood in the various tubes with culture medium. In order to elucidate this problem, blood was withdrawn into citrate venules from 50 normal persons. Prior to disinfection of the skin — as done in most hospital wards, simply by washing with ether — cultures were made from the skin of the cubital fold. For these cultures, a cotton swab, moistened with sterile saline, was rubbed over the skin, and a blood agar plate was inoculated with the swab. The technique of distribution of the citrate blood was the same as usual. The outcome of the skin cultures is recorded in Table 3. Here, as will be noticed, staphylococcus and coryneiform bacteria were by far the most common bacteria of the skin. In one case the blood culture yielded growth of staphylococcus albus in 1 out of 16 tubes, and the same microbe was demonstrated in the skin culture (numerous colonies) from this person. In this case, presumably, the blood culture was contaminated from the skin. The remaining 49 blood cultures showed no growth. So contamination from the skin ought to be avoidable by sufficient care in the disinfection of the skin and withdrawal of the blood.

Table 4 gives the percental frequency of bacterial growth in the five culture media here employed, covering merely the more frequently isolated bacterial groups. In cultivation of the bacteroid group the cysteine-containing media (anaerobic) proved far superior to the others. There is no striking difference in the results obtained with serum broth, Truche's medium and semifluid agar. Still, Truche's medium appears to have been more suitable than the others for culti-

Table 2.

Combined Bacterial Findings in 1, 2 or more Specimens of Blood from the Same Patient.

27 patients	yielded	staph. albus	and	coryneif. bact	in the same blood cult.
2	»	»	»	»	Bac. typhi » » » » »
1	»	»	»	»	Gram-neg. rods » » » » »
1	»	»	»	»	pneumococcus » » » » »
1	»	»	»	»	staph. aureus » » » » »
1	»	»	non-hem. strept.	»	colon-like bact. » » » » »
1	»	»	staph. aureus	»	Bac. coli » » » » »
1	»	»	coryneif. bact.	»	Gram-pos. coccobact. » » » » »
1	»	»	staph. albus	»	coryneif. bact. and staph. aureus » » » » »
			<i>in 1' blood culture</i>	<i>in 2' blood culture</i>	<i>in 3' blood culture</i>
2	»	»	staph. albus and coryneif. bact.	coryneif. bact.	coryneif. bact.
1	»	»	staph. albus and coryneif. bact.	staph. albus	
1	»	»	staph. albus and Bac. typhi	» »	
1	»	»	non-hem. strept. and Gram-neg. rods	non-hem. strept.	non-hem. strept.
6	»	»	staph. albus	» » »	
3	»	»	» »	coryneif. bact.	
2	»	»	» »	staph. aureus	
1	»	»	» »	colon-like bact.	
1	»	»	» »	staph. citreus	
1	»	»	» »	Salmonella Dublin	
1	»	»	staph. aureus	antracoid bact.	
1	»	»	coryneif. bact.	meningococcus	
1	»	»	» »	staph. aureus	
1	»	»	» »	non-hem. strept.	
1	»	»	» »	Gram-neg. cocci in clusters	
1	»	»	Bac. typhi	sarcina	
2	»	»	staph. albus	hemolyt. strept.	hemolyt. strept.
1	»	»	» »	non-hem. strept.	non-hem. strept.
1	»	»	» »	staph. aureus	staph. aureus
1	»	»	non-hem. strept.	pneumococcus	pneumococcus
1	»	»	» » »	Bac. coli	Bac. coli
1	»	»	staph. aureus	non-hem. strept.	non-hem. strept.
1	»	»	coryneif. bact.	» » »	» » »
1	»	»	staph. albus	and in 3 later cultures:	non-hem. strept.
1	»	»	coryneif. bact.	» » 3	» » »
1	»	»	staph. albus	» » 4	» » »
1	»	»	coryneif. bact.	» » 4	» » »
1	»	»	» »	» » 4	» » »
					once, Gram-neg. rods once, and staph. aureus twice.
1	»	»	staph. albus	» » 5 later cultures:	non-hem. strept.
1	»	»	staph. aureus	» » 8	» » » » »

77 patients

Table 3.
Results of Skin Cultures from 50 Normal Persons.

Bacterial species	Number of colonies					Number of persons
	Countless	Numerous	Several	Few	Very few	
Staphylococ. albus	7	15	16	8	2	48
Coryneiform bacteria		7	11	3		21
Staphylococ. citreus			4	2	4	10
Sarcina		1	3	3	3	10
Gram-negative rods	1	2	1	1	1	6
Bac. subtilis			1	1		2
Staphylococ. aureus			2			2
Micrococ. catarrhalis			1	1		2
Non-hemolytic strept.					1	1
Gram-neg.cocci in chains			1			1

Table 4.
Percentage of Growth in the Culture Media Employed.

The upper figure in each set gives the number of inoculated tubes containing the respective culture medium in the cultures showing growth in one or more media.

	Serum broth	Truche's medium	Semifluid agar	Cystein agar	Cystein broth
Staphylococ. albus	1510 18%	1510 38%	1501 29%	493 23%	1508 13%
Non-hemolytic strept.	1261 37%	1261 62%	1239 47%	575 47%	1266 53%
Staphylococ. aureus	452 46%	452 60%	451 63%	370 29%	451 41%
Coryneiform bacteria	212 9%	212 13%	213 16%	26 4%	212 7%
Hemolytic streptococci	176 60%	175 63%	174 53%	84 68%	175 58%
Pneumococci	110 77%	109 68%	109 68%	52 81%	109 60%
Typhoid bacilli	85 21%	83 27%	83 21%	35 29%	86 20%
Bacteroids	88 13%	88 5%	86 27%	49 61%	86 85%
Colon-like bacteria	53 55%	53 49%	52 54%	32 41%	64 28%
Colon bacilli	36 61%	36 67%	36 78%	13 54%	36 58%
Sarcina	26 4%	26 15%	26 31%	0	26 4%
Staphylococ. citreus	24 33%	24 46%	24 50%	8 50%	24 21%
Meningococci	26 27%	26 54%	26 35%	4	26 15%
Paratyphoid bacilli	13 16%	13 31%	13 31%	6 67%	13 31%
Bac. subtilis	20 0	20 10%	20 15%	0	20 5%

vation of non-hemolytic streptococci. *Staphylococcus albus*, coryneiform bacteria, typhoid bacilli, sarcina and *B. subtilis* gave growth on the whole only in relatively few tubes, whereas hemolytic streptococci, pneumococci, colon bacilli and bacteroids most often gave growth in many tubes, *i. e.*, massive growth.

Table 5.

Number of Positive Blood Cultures within Each Bacterial Group arranged after the Number of Tubes showing Growth.

	Number of tubes with growth															
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
<i>Staphylococcus albus</i>	134	83	50	39	19	23	10	23	13	9	5	7	9	4	1	18
Non-hemolytic streptococci	19	14	12	21	12	15	8	5	7	18	6	4	7	4	5	30
<i>Staphylococcus aureus</i>	20	15	4	13	5	8	3	9	5	3	2	8	7	5	3	25
Coryneiform bacteria	34	14	4	5	1	1	2									
Hemolytic streptococci	4	7	2	2	2	2	3	1	2	2		3	2	2	4	15
Pneumococci	9	4	3	6	1		2	1	2		5		1			16
Typhoid bacilli	4	2	6	2	2	3		2			1					
Bacteroids	1	4	3	5	1	3	1	1	1	2	1					
Colon-like bacilli	1	2		2	2		1	1	1			1		1		2
Colon bacilli	2			2				1	1							5
Sarcina	3	4		2												1
<i>Staphylococcus citreus</i>	1	1	2	1	1						1		1			
Meningococci	1	2			1	2		1		1						
Paratyphoid bacilli	1		2									1				
<i>Bac. subtilis</i>	4	1														
<i>Bac. proteus</i>	1		1	1	1											
Gram-neg. coccobacilli							1				1					
<i>Bac. pyocyaneus</i>		1							1			1				
<i>Bac. influenzae</i> Pfeifferi												1				
<i>Bac. anthracis</i>																1
<i>Bac. phlegm. emphysem.</i>												1				
<i>Salmonella</i> Dublin	1															
<i>Salmonella</i> Oranienburg						1										
Gram-neg. cocci in chains			1													

Table 5 gives the numbers of positive cultures within each bacterial group, the first column covering the cultures in which growth was obtained only in one tube, the second column covering the cultures with growth in two tubes, and so on. *Staphylococcus albus* and coryneiform bacteria are seen to have grown most often only in one tube or two, while many of the other bacteria just as often gave growth in more than half of the tubes.

Naturally it is of interest to see in which morbid conditions the blood may be expected to yield bacterial growth. Table 6 gives a survey of the clinical diagnoses most often put down on the cards received together with the venules. In each case, thus, the clinical diagnosis recorded was made before the hospital received a report from the institute about the outcome of the blood culture. So these

diagnoses are to be accepted only with a certain degree of reservation. The figures to the right in each column give the number of cultures with growth in at least one-half of the tubes. The diagnoses and bacterial species are entered after their relative frequency in this material, showing that febrilia and sepsis are by far the most common diagnoses. *Staphylococcus albus*, coryneiform bacteria, typhoid bacilli and colon-like bacteria were encountered more often under the diagnosis febrilia, *staphylococcus aureus* and hemolytic streptococci more often under the diagnosis sepsis. The non-hemolytic streptococci occurred most often under the diagnosis endocarditis, the pneumococci quite preponderantly under the diagnosis pneumonia, and the bacteroids especially under the diagnosis angina (sore throat).

Table 7 gives the intervals from the onset of illness — as recorded on the cards of the patients — to the withdrawal of the blood yielding the first positive culture. The first figure in the upper row of figures for each bacterial groups gives the number of patients with positive cultures who had been ill for up to one week; the second figure in the same row gives the patients who had been ill for more than one week but not over two weeks, and so on. The column to the extreme right covers patients who had been ill for six months or more. The lower row of figures for each bacterial group gives the number of patients with cultures showing growth in at least one-half to the tubes. In particular from patients infected with pneumococci was positive venule blood received early in the disease. The same applies to cases in which *staphylococcus aureus* and hemolytic streptococci were demonstrated in the blood. From patients infected with non-hemolytic streptococci the venules yielding bacterial growth were often received much later in the course of their disease, probably because the clinical symptoms in the respective cases were not alarming. Undoubtedly, examination by blood culture should not be restricted to cases in which a positive result may be expected but rather be employed, preferably repeatedly, in all cases with febrile phenomena of unknown origin.

It is only reasonable, as mentioned, to reckon that the bactericidal properties of the blood will assert themselves in venules and blood cultures; hence it has been recommended to add active sterile trypsin or liquoid to the blood in order to abolish or reduce the bactericidal faculty of the blood. Besides the addition of trypsin or liquoid should also inhibit coagulation of the blood. In experiments carried out by the writer it was not practicable to prevent the clotting of the blood by addition of an equal part of trypsin solution with pH varying from 6.5 to 8.5. Still, with $\text{pH} = 8$, clotting was inhibited for up to 70 min. Liquoid, on the other hand, inhibited the clotting completely.

The trypsin solution here employed was prepared by adding 1 % trypsinum purum to distilled water. The suspension was left standing

Blood Cultures, and the Bacteria grown in the Respective Cases.

[illegible]

at room temperature for a couple of hours, being shaken repeatedly, then filtered sterile through Berkefeld filter.

In order to see what effect the bactericidal faculty of the blood might have on various bacteria under different conditions the following experiment was performed.

20 cc. of human blood was distributed equally on 3 flasks containing 1.5 cc. of one of the following three solutions: 1) 3.8 % sodium citrate; 2) a solution of trypsin in 3.8 % sodium citrate solution prepared in the same manner as the above-mentioned trypsin solution, but with employment of sodium citrate solution for solvent.; 3) 1 % liquoid solution. From the flasks, 1 cc. of the mixture was transferred to test tubes. 7 bacterial strains of different species were used in the experiment. The bacteria were suspended in trypsin broth, and the suspensions were standardized and diluted to a suitably slight density. With a special platinum loop of 0.03 cc. capacity, the tubes were inoculated: three different tubes with the same microbe. Further, the density of the trypsin suspensions was ascertained by inoculation of blood agar plates and subsequent counting of the colonies. The tubes were incubated at 37°; and after 6, 16, 24 and 48 hours' incubation the bacterial density of the mixtures was determined by counting the colonies on the blood agar plate, which was divided into three equal fields, and each field was inoculated with 1 loopful (0.03 cc.) of the mixture. The results are recorded in Table 8.

The addition of liquoid to the blood results in a far better growth of the bacteria in the blood than is obtained when sodium citrate or trypsin citrate is employed. It is reasonable, therefore, to assume that better cultural results may be obtained by employment of liquoid venules instead of citrate venules.

Summary.

On routine cultivation from specimens of blood withdrawn in citrate venule, bacterial growth is obtained in 17.8 % of the 8799 blood specimens examined. The cultural method employed and the bacteriological findings are mentioned in detail.

Further, the clinical diagnoses made on the patients yielding growth of bacteria are recorded.

Experimental evidence is presented to the effect, that various bacteria grow better in liquoid blood than in citrate blood.

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STUDIES ON CONGENITAL SYPHILIS DIAGNOSED IN THE PERIOD OF 1935—1939*)

I. DIAGNOSIS OF CONGENITAL SYPHILIS

By *Peter Krag and Esther Dalsgaard-Nielsen.*

(Received for publication Jan. 18th 1944.)

In 1940 Krag & Lindhardt**) showed that there was a pronounced disagreement between the number of new-diagnosed cases of congenital syphilis obtained on summation of the weekly reports from the National Health Department and the cases registered in the syphilitic card index of the State Serum Institute, the latter series of figures lying at a considerably higher level for all age-classes.

As, furthermore, a rough comparison of the age distribution for the figures of the Serum Institute and for the materials reported in Dalsgaard-Nielsen's dissertation***) showed a systematic age difference (relatively few patients over 20 years in the dissertation), we found it reasonable to try to throw some additional light on the occurrence of the new-diagnosed congenital syphilis.

The starting material is a syphilitic card index of the State Serum Institute, in which the following categories of patients are registered as new cases:

1. All patients who for the first time give one or more positive serum reactions, regardless of the strength.

2. The seronegative patients about whom the information is obtained that syphilis is diagnosed clinically or is highly probable.

For all seropositive patients information is obtained by questioning about the clinical diagnosis and syphilitic symptoms (in 1935 and 1936, however, this took place only in cases of specimens received from hospitals and clinics in Copenhagen).

*) We wish to acknowledge our appreciation of the stimulating and instructive discussions of certain problems in this work with Mr. G. Rasch, Ph. D. and Mr. E. Hald, Actuary.

**) p. 291.

***) p. 289 in this paper.

Translated from Danish by Hans Andersen, M. D.

On the basis of this information a list of the new registrations was worked up every month, and at the same time each case was classified as: acquired syphilis (Stages 1, 2 and 3, cardiovascular and cerebrospinal syphilis), congenital syphilis, or entirely serodiagnostic syphilis.*)

For each year in the period of 1935—39, from these lists we picked out the unquestionable and the probable cases of congenital syphilis (altogether 406 cases, see Table 1) as in order to include everything of possible interest we have entered on the list also a number of seronegative patients about whom their physician in 1935—36 reported: »Congenital syphilis diagnosed today«, »syphilitic deformity of the teeth«, »keratitis«, etc. For each of these 406 patients, all the available data were tabulated (marks of identity, sometimes the name, clinical features, serological course, and sometimes information about the family. After this, fairly detailed questionnaires were sent to one or more of the physicians who, according to the card index, have examined (and treated) the respective patients.**)

The 406 patients of the total material were tabulated after year and day of birth and then given consecutive numbers, the lowest numbers being given to the oldest patients. After the return of the questionnaires, the total material could be divided into several groups after the certainty of the syphilis diagnosis. A separate group was made up of all the cases in which it was quite certain that congenital syphilis was diagnosed serologically within the period of 1935—39 (see the 304 cases designated as a_1). Then the rest of the material was divided into 8 groups: a_2 , a_3 , b, c, d, e, a_3/c and b/d . A review of these 102 cases will show how strict requirements we have stipulated for the group »unquestionable new-diagnosed congenital syphilis«.

From Table 1 it will be noticed that this more detailed enquiry has yielded some new information of various nature:

Group a_2 shows that 12 cases were disclosed as having been diagnosed previously even though the physician of the respective patients some years before had not known this fact or had not considered it necessary to let us know about it.

Of these 12 patients, 8 are over 30 years old, and in 11 cases the first diagnosis of syphilis was made before the patient was 16, as a rule because of the presence of keratitis (9 patients). 2 of these patients were found in the syphilitic card index a few years prior to the rediscovery of the illness, but these specimens of blood had been provided with an erroneous date of birth. Further mention of these 12 patients appears to be of no interest in this work.

* Besides, we have made use of various designations for the entirely serologically diagnosed cases corresponding to the value of the serum diagnosis. Finally, there are also groups for suspect seropositive and seronegative cases.

** For the thorough information given in our detailed questionnaire we are greatly obliged to our colleagues, in hospitals as well as in general and special practice.

Table 1.
Survey of Classification of the Starting Material.

	No. per year					Total	Percental age distribution (years)		
	1935	1936	1937	1938	1939		<1	1—19	≥20
a ₁ . Unquestionable congenital syphilis, new-diagnosed	56	42	81	68	57	304	24.0	59.2	16.8
a ₂ . Unquestionable congenital syphilis but diagnosed prior to 1935	3	5	2	1	1	12		33.3	66.7
a ₃ . Unquestionable syphilis probably congenital		1	3		2	6			(100.0)
b. Doubtful syphilis, unquestionably congenital	1	6	3	2	2	14	64.3	14.3	21.4
c. Unquestionable syphilis acquired		1		3	3	7		(42.8)	(57.2)
d. No syphilis	9	9	2	4	3	27	63.0	25.9	11.1
e. Insufficient information	5				1	6	(50.0)	(33.3)	(16.7)
a ₃ /c Unquestionable syphilis but uncertain whether congenital or acquired	2	2	5	2	10	21			100.0
b/d Perhaps congenital syphilis / perhaps no syphilis at all	4	2	1		2	9	(33.3)	(33.3)	(33.3)
Total	80	68	97	80	81	406	The figures in brackets are calculated on the basis of observations ≤ 10.		

Group a₃ (unquestionable syphilis, probably congenital) comprises 6 patients, all over 20 years.

Of these patients 3, aged 50—68 years, gave rather weak but unquestionable serum reactions, and all presented remnants of a past keratitis —

two of them also bone and nerve symptoms. The remaining three patients were 20—25 years old and showed a strong serum reaction, but only one of them presented possible symptoms of congenital syphilis (bilateral deafness, which partly is described as syphilitic neurolabyrinthopathy, partly put down as related to the deafness of the mother and sibs. These relatives were not examined by means of the Wassermann test; the child of the patient has congenital syphilis; see a subsequent section, V, Pt. No. 287, syphilis in 3' generation). Of the remaining two patients, one has a syphilitic mother, while both parents of the other are syphilitics.

That we have considered the diagnosis of congenital syphilis somewhat uncertain in these 6 patients illustrates the strictness of our diagnostic requirements, so that there may hardly be any doubt as to the correctness of the diagnosis in the 304 patients in whose cases anamnestic data and clinical features are more weighty evidence.

Group b comprises 14 patients. Here the diagnosis of syphilis is somewhat doubtful as either the serum reaction or the symptoms are uncertain. Common to all these patients is the condition that: if they have syphilis it certainly is of congenital origin. These patients may be divided into two subgroups:

1. 9 patients were all under one year of age and all had a syphilitic mother. Of these patients 3 died at the age of 1, 1 and 6 months respectively, without receiving antisyphilitic treatment; the serum reactions were rather weak and the symptoms atypical (1 WR negative, premature child with bullous dermatitis, admitted to hospital on 18' day of life and died two days later; and 2 symptom-free children with serum reactions: $WR = 2$, $KR = 1$ *) and seropositive mothers; both children died before the test could be repeated; the cause of death was respectively premature birth and bronchopneumonia).

One of the six living patients presented no symptoms, and the serum reactions ($WR = KR = 3$) subsided spontaneously in 5 months.

The last 5 patients were all given some form of antisyphilitic treatment. The serum reaction subsided in less than half a year, even in two cases, in which both WR and KR were ≥ 4 ; these two specimens consisted in blood from the umbilical cord, and the seronegativity was ascertained already on repetition of the test at the age of 2—3 months. (In both cases the syphilis of the mother was acquired and diagnosed during the pregnancy, and un-

*) In this and the following papers the various serum reactions are designated by the following abbreviations:

Wassermann's reaction	WR
Kahn's	KR
Presumptive Kahn	PK
Müller's Ballung	MB
Meinicke's Klärung	MR

WR, KR are given in degrees of strength — a logarithmic expression for the titer (1). PK, MB and MR are given as strong (++), weak (+), doubtful (\pm). As to the specificity and sensitivity of these reactions the reader is referred to previous papers in *Acta dermato-venereolog.* (2, 3) and *Ugeskrift for Læger* (4, 5).

The total result of WR and KR or of all 5 reactions are recorded as +, \pm or — (cf. 2, p. 528).

doubtedly the fear of neglecting some useful measure has been an essential indication for the treatment given. Otherwise the general rule is to observe the symptom-free seropositive children, as in several cases the reaction is merely transmitted from the mother.) Examination of blood from the umbilical cord is only of slight significance: a negative reaction does not exclude a subsequent + WR, and a positive reaction may be transmitted from the mother, constituting thus merely an indication for continued observation. Furthermore it is to be emphasized that in a few cases a positive reaction of the umbilical cord blood is seen to disappear spontaneously, altogether or partially, whereafter the child 1—2 months later gives a true and strong serum reaction, sometimes in connection with a skin eruption.

Of the 5 last-mentioned mothers 2 had gone through 2 and 6 pregnancies, respectively, without having any viable child. In both cases the diagnosis was made on the basis of WR, which in one case was verified by the autopsy findings (syphilitic liver lesion) in the child. In the other case the examination was considered indicated when the long case history of the patient was told to her new physician. Antisyphilitic treatment during the subsequent pregnancy resulted in the presumably healthy children entered in Group b, who for the sake of safety both were given a relatively short treatment.

2. The 5 remaining patients in Group b were 11—35 years old. None of them gave a strong typical serum reaction; and the anamnestic-clinical data were as follows: 1) Brother suffering from congenital syphilis; the patient died of purulent meningitis following otitis media, spinal fluid WR = 2. — 2) Both parents syphilitic, brother showing \pm WR; the patient presenting loss of hair, WR = 0, KR = 1, subsiding spontaneously. — 3) Family history lacking; the patient presented keratitis and suspected deafness, but presumptive WR was negative. — 4) The mother is syphilitic; the patient showed sequelae of keratitis, enlargement of the spleen, a few positive precipitation reactions, and became seronegative after moderate treatment. — 5) Family history lacking; relapsing hydrarthrosis of the knee and typical serum reactions; both symptoms subsided after a brief series of neosalvarsan + bismuth.

Group c (unquestionable acquired syphilis) comprises 7 patients: 1 of 18 months and 6 aged 17—54 years.

The peculiar instance of a child, 18 months old, suffering from acquired syphilis is to be mentioned by itself: the mother is said to have given a negative WR at the time of delivery, 31/5/35. In November 1936 a fresh syphilitic infection was diagnosed in the mother (macular and papular syphilides, leucoderma and + WR). At the same time the child presented macular syphilides, enlargement of peripheral lymph glands, enlargement of the spleen and unquestionably positive WR, which yielded to specific treatment in the course of 1 year.

The 6 adult patients gave all WR and KR = 6. The information first received was suggestive of congenital syphilis: 2 cases of periostitis in patients aged 17—20 years; 1 case of keratitis; 2 imbecile without any information about the possible source of infection, and 1 patient presented an eye lesion in childhood. A subsequent, more detailed, inquiry yielded the information that the first 3 of these patients showed definite signs of fresh acquired syphilis; in the cases of the 2 imbecile patients there was a considerable chance of acquired syphilis, while the two mothers probably were healthy; and in the last patient the eye lesion was found presumably to be post-diphtheric.

The first 3 adult patients became seronegative in less than 1 year — something which highly supports the diagnosis of acquired syphilis. In the remaining 3 cases, information is wanting as to treatment and cure or later serum reactions.

Group d comprises 27 patients for whom the diagnosis congenital syphilis (or suspect congenital syphilis) was later refuted.

Of these patients 17 were under 1 year; 4 were 1—9 years old, and 6 were over 10 years. 10 patients (including 5 who were over 6 years) were *seronegative*, and the primary information furnished by the physician about congenital syphilis was refuted at once by questionnaire; 9 of these patients were registered in the syphilitic card index in 1935—36.*)

Only 4 of the patients gave serum reactions corresponding to the total result +; they were all free from symptoms: 1 was a woman, aged 35, with $WR = KR = 1$, corroborated by all three amplified reactions. This reaction was obtained while the patient was hospitalized for extrauterine pregnancy, and it disappeared spontaneously about one year after the operation. 3 patients were children of syphilitic mothers; the reaction was found in the umbilical cord blood, and it subsided spontaneously in two of these children, while the third was given antisyphilitic treatment immediately after the blood examination. After two series of calomel, this patient was transferred to the Welander Home, where the treatment was discontinued as the blood sample from the child, who was now $3\frac{1}{2}$ months old, turned out negative in all five tests. After this, the patient was under observation for two years and was continually seronegative and symptom-free.

The remaining 13 patients gave weak WR and/or KR (total result \pm).

Of these patients 9 were symptom-free infants whose serum reaction subsided soon and spontaneously (reaction transmitted from the seropositive mothers).

For the last 4 patients in Group d the suspicion of congenital syphilis was given up after the serological observation, without institution of any treatment. The data in these four cases are as follows: 1) Patient, 19 years old, suffering from pulmonary tuberculosis; $WR = 3$, $KR = 1$; seronegative 24 days later; sibs stated to be well. 2) Patient, 5 years old, with uncertain rash; $WR = 1$, $KR = 0$; on amplified test, 10 days later only MB +; mother seronegative, whereafter suspicion of congenital syphilis was given up by the hospital and by us. 3) Patient, 2 years old, with impetigo; $WR = 4$, $KR = 0$; seronegative on 12' day; examination indicated exclusively by the presence of a syphilitic heart lesion in the father. 4) Patient, 2 years old, deaf, without signs of syphilis; $WR = 1$, $KR = 2$; one year later, seronegative; no information about the family.

Group e (insufficient information) comprises 6 cases, including 3 with strong serum reaction. Detailed information about these cases could not be obtained because the respective physicians had died in

*) A review of the monthly lists of the card index for these 2 years was the cause of restriction of the indications for registration; at the same time a new case-record card was adopted with more exact questions (the original question: »May it be a case of congenital syphilis?« implied a certain source of error, as many physicians would answer in the affirmative also when the specimen of blood came from a patient who merely was under observation for congenital syphilis, and at that time these specimens had to be registered as seronegative cases of possible congenital syphilis).

the meantime or quit practising, or because of defective case records or due to the general difficulty in a name or address card index to find patients who are known only by initials and date of birth. In the case of hospitalized patients it may often be taken for granted that the specimen of blood was withdrawn shortly after admission, and thus the name of the patient in question may often be found through the admission register — but this adjuvant fails completely when it comes to locate a patient in the archives of practising physicians. In this way too, perhaps, 4 cases of congenital syphilis have been lost to the materiel.

Finally, it was found necessary to set up two transitional groups: a_3/c and b/d .

Group a_3/c . In several cases of adult patients it is impracticable to decide whether the syphilis is acquired or congenital — as, for instance, in the following case: Woman, 23 years old, without clinical signs of syphilis — the serum reaction was discovered accidentally — parents died of pneumonia and after an accident — no sibs — the patient denies any knowledge about having syphilis. Now, if her denial appears plausible, the physician will be inclined to answer the question with »congenital syphilis probable«. In the present work, however, this judgment is *not* considered sufficiently weighty to designate the patient as belonging to group a_1 (unquestionable congenital syphilis).

This group comprises 21 patients, aged 20—66 years, whose serum reactions corresponded to the total result + (except in the case of one patient, 42 years old, with weak MB and doubtful MR; the patient was mentally defective and had perforation of the nasal septum; no information about the family).

Only 7 of the 20 patients with total result + presented symptoms which slightly or decidedly indicated the presence of syphilis: optic neuritis at the age of 23 years — doubtful dental deformity — cerebrospinal syphilis — arthritis gen. — WR negative in the given source of infection; half a year prior to the examination, a dotted rash which on the upper part of the trunk has left a macular brownish pigmentation (elements of pea size; 4 sibs died in infancy; father syphilitic for many years — tertiary syphilide — tiredness — in only the last two cases was information obtained about the mother — in both cases, syphilis of long standing.

Of the 13 symptom-free patients, 8 gave definite information about syphilis in the mother; one was uncertain about syphilis in the mother but positive in the father. In the remaining four cases no information was obtained about the family at all, and the suspicion of congenital syphilis was based merely on the above-mentioned judgment of the physician.

Finally, *Group b/d* comprises the very uncertain cases in which we had to consider whether it was a question of congenital syphilis or perhaps not syphilis at all.

This group includes 9 patients, 3 of whom were under one year, 3 were 10—20 years, and 3 over 29. In 3 patients the serum reaction was negative;

these patients presented a parenchymatous keratitis or remnants of such a lesion, but otherwise no anamnestic-clinical symptoms (cf. IV, on serum reactions in new-diagnosed parenchymatous keratitis):

In two infants with a transitory rash the total result of the serum reactions was \pm ; only one of the mothers had syphilis. Both of the children were treated with myosalvarsan, and on repetition of the test the serum reaction was negative respectively 3 and 8 months after the suspicion arose.

In the four last cases the total result of the serum reaction was +. In one of these cases the test was made on umbilical cord blood (WR = 4, KR = 5); the reaction subsided spontaneously, as ascertained 1½ years later; the mother was syphilitic, and two sibs gave transitory \pm reactions. The three other patients were 16—18 years old, presenting respectively a doubtful leucoderma, headache, and findings suggestive of pulmonary tuberculosis — pityriasis rosea? Infiltration of the lungs. — Tuberculosis of the nasal septum? Improvement on treatment with potassium iodide.

The mothers of the first two of these patients were seronegative; the patients were given antisyphilitic treatment and became seronegative in ≤ 3 months. Presumable, then, the reaction in these cases was unspecific or due to acquired syphilis.

In the case of the third patient the only information was that the father had + WR. In January 1942 a specimen of blood from this patient was again received (WR = 12, KR = 10) together with this information: No anti-syphilitic therapy since the above-mentioned treatment with potassium iodide; the patient now presents a saddle-nose and a large ulceration of the soft palate.*) Antisyphilitic treatment of this patient with neosalvarsan + bismuth is now instituted (February 1942: WR = 15, KR = 12).

On analysis of our 5-year material with reference to the above-mentioned Groups a₁-b/d, the unquestionable congenital syphilis is seen to have been relatively infrequent in 1935—36. This may perhaps be due to the circumstance that several cases of congenital syphilis were not designated as such by the respective physicians; and the natural limitation of the number of questionnaires has prevented us from isolating such cases among the many cases of new-discovered positive serum reaction in which the given information has been rather defective. The age distribution of the patients shows, however, that the low frequency is found in all age-classes.

Further, the first two years have rather many cases of non-syphilis or doubtful syphilis; this is due to the technical conditions of the registration.**)

Finally, the last two years of the period here concerned have an excess of cases of acquired or possibly acquired syphilis, as in 1939 (temporarily) not only unquestionable cases but also doubtful cases were classified as congenital syphilis. Revision of the classification by means of the information obtained through the detailed questionnaire

*) These data were received too late for transfer of the patient from Group b/d to Group a₁.

Owing to an error in our lists that was discovered too late, we further have missed a symptom-free patient, aged 22, a brother of two unquestionable cases of congenital syphilis (Nos. 78 and 97).

**) See foot-note on p. 269.

has shown that it is not practicable with certainty to establish the presence of congenital syphilis in these primarily suspect cases.

In 1939 Krag & Lindhardt reported that the syphilitic card index in 1937—38 had registered 173 new cases of congenital syphilis, whereas summation of the weekly health reports gave only 115 cases.

Revision of the cases in the syphilitic card index leaves 154 quite unquestionable cases, 3 highly probable and 5 doubtful — 162 altogether — so that the conclusions advanced at that time by no means need modification, in particular as at that time it was demonstrated that the weekly reports occasionally included notified cases of such a doubtful character that were not reckoned even in the aforementioned figure (173). (For further details, see the following paper.)

Review of the Material Proper: 304 Patients with New-discovered Congenital Syphilis.

In the following an account will be given of the classification of the symptoms, together with a brief survey of the clinical features implied by the individual group of symptoms.

For each patients all the symptoms are reckoned that have helped the authors to establish the diagnosis congenital syphilis, regardless of whether or not these symptoms were present as acute phenomena when the patient's physician made his diagnosis. In a later section (III p. 298) an account will be given of the symptoms present at the very time of the diagnosis. For illustration of the symptoms reckoned here, we may mention the later appearance of dental deformity or mental deficiency in a child whose syphilis was diagnosed at the age of two months, or a patient with remnants of a past keratitis.

Symptom 1 comprises all skin symptoms. In our material altogether 48 patients presented or had presented skin symptoms of specific nature. As was to be expected, these symptoms were found especially in the youngest part of the material, 38 out of the 48 patients being under 1 year (including 9 newborn). The sex distribution was equal, with 26 males and 22 females. The most frequent form of skin eruption was papular exanthema, this being found in 18 patients; rhagades and fissures were present in 11 cases, specific ulcers in 2, and unspecified skin lesions in 5. The remaining 12 patients presented various phenomena as: undefined exanthema, desquamation, infiltrations, dermatitis, etc. In all the last-mentioned 5 + 12 somewhat uncharacteristic or vaguely characterized cases the diagnosis congenital syphilis was corroborated by the serum reaction or by other symptoms of congenital syphilis. In 2 infants, however, the »supporting evidence» consisted merely in maternal syphilis combined respectively with congenital syphilis in a brother and Symptom 10 in the patient (small atrophic child who died of capillary bronchitis). The diagnosis is considered unquestionable in all 48 cases.

At the time when the diagnosis was made, 5 of these 48 patients presented no actual skin symptoms but definite data on past phenomena of this character: skin eruption in infancy, scars after previous ulcerations or fissures.

Symptom 2 comprises phenomena localized to the mucous membranes. Also in this group, which comprises altogether 38 patients, the youngest age-class made up a great majority, 26 of the patients being under 1 year. The sex distribution was: 22 males and 16 females. Coryza was by far the most frequent symptom. All the 26 infants had coryza. 5 children were 2—10 years old; 4 of them had often coryza, sometimes protracted; 1 had perforation of the soft palate. The age group of 14—40 years comprises 7 patients: 2 with glossitis and fissures, 1 with ulceration of the palate and tonsil, and 4 with nasal affections (rhinitis, slight degree, improved on antisyphilitic treatment; — coryza and hypertrophy of the inferior turbinate; — coryza, improved on antisyphilitic treatment; — atrophic syphilitic rhinitis). In the first two of these four cases the diagnosis of syphilis is supported merely by strong serum reactions and information about syphilis in sibs and in the father and mother, respectively. (The last two patients presented several definite clinical signs of congenital syphilis.)

Symptom 3 covers syphilis involving internal organs, *i. e.*, liver and spleen. This group comprises only 17 patients, 9 males and 8 females; 16 of them were under one year. In 9 of the cases only the liver was enlarged, while the remaining 8 patients presented enlargement of both the liver and the spleen. One patient was a man, 18 years old, with enlargement of the liver, dulness, choroiditis, bilateral hydrarthrosis of the knee, besides slight ataxia; he gave a strong positive serum reaction. Even in the absence of any information about his family, the diagnosis congenital syphilis is unquestionable.

All the patients presenting one or more of Symptoms 1, 2 and 3 are given the joint designation: Symptom A. Altogether 68 patients presented this symptom complex, and two-thirds of these patients were under one year. Besides the mentioned cases in which internal organs and bones were involved, it has to be mentioned that autopsy on 3 symptom-free infants revealed two instances of cirrhosis of the liver and one of osteochondritis (respectively No. 302, died 45 min. after birth; No. 330, died 3 months after birth; and No. 389, died 3 days after birth).

Symptom 4 comprises 76 patients with parenchymatous keratitis,*)

*) In 1941 a symptom-free treated boy (No. 368), aged 3 years, had keratitis (not included here under keratitis).

together with a boy (No. 288), one year old, with glaucomatous iritis and a woman (No. 111), aged 19, with iridocyclitis. Undoubtedly the boy had a syphilitic eye lesion, the chance of another etiology being rather slight (serum reaction: WR = 15, KR = 12, amplified: strong). The woman presented a slight parenchymatous keratitis, her case record stating, among other things, that the periphery of the cornea cleared up under the treatment (serum reaction: WR = 8, KR = 7). Of the keratitic patients, however, 16 had gone through the acute phase of the disease without getting in contact with the syphilitic card index and without receiving any antisyphilitic treatment.

As to the age distribution of the patients with parenchymatous keratitis, the reader is referred to a comparison between the material from Dalsgaard-Nielsen's dissertation and the present material which will appear in the following paper II, Table 3.

The serum reactions of the keratitic patients will be mentioned especially in a later paper (IV p. 312).

Symptom 5 comprises bone changes, especially periostitis and saddle-nose. Altogether 28 patients are found to present such symptoms, 14 males and 14 females, 11 of whom were under one year, while 3 were 1—9 years old, 9 were 10—19 years, and 5 were 20—44. Saddle-nose was present in 8 cases, periostitis in 9. Further, deformity of the palate was found in 3 cases, and osteochondritis in 4. Finally, the following symptoms were each observed in one case: dactylitis; sequelae after osseous affection in childhood; cranial deformity (steep brow); undefined bone lesion. Each of the four last patients presented 1—2 unquestionable clinical signs of syphilis. Two of the patients with osteochondritis presented Parrot's pseudoparalysis.

In the patients under one year the symptoms were: osteochondritis (4), periostitis (5), deformity of the palate (1), and dactylitis (1).

Symptom 6 covers joint affections. This group comprises 28 patients, 14 males and 14 females. The age distribution of these patients was as follows: 5 were 4—9 years; 13 were 10—19 years; 6 were 20—29 years; and 4 over 30. The predominant symptom was arthritis or hydrarthrosis of the knee, being present in 23 cases. In the remaining 5 patients the symptoms were less typical: one man (No. 159), aged 17 had a mouse in the knee-joint; microscopy showed syphilitic osteochondritis. Two women, aged 25 and 35, gave a past history of »rheumatic fever« in childhood; now the character of this lesion cannot be cleared up. The last two patients were a woman, aged 58, with arthritis of the wrist and knee-joint, and a boy, aged 14 years, with periostitis of the humerus. In the last-mentioned four cases the diagnosis congenital syphilis is based on coincident unquestionable symptoms except in the last case; here the positive evidence consists merely in the serum reaction and a family history

of syphilis in the mother and sibs. In 8 of the total 28 cases the joint lesion dated back several years in the history of the patient.

Symptom 7 covers ear symptoms of specific nature. This group comprises 13 patients, 5 males and 8 females. As was to be expected, from previous accounts, the patients were older in this group than in the others, the age distribution being: 5 patients of 10—19 years, 2 of 20—29 years, 3 of 30—39 years, and 3 of 40 years or more. The case records furnish no thorough information. In 5 cases it is merely stated that the patient was deaf, in 2 cases that the hearing was slightly impaired, while in 1 case a diagnosis of otosclerosis (syphilis) was recorded, and in 5 cases there was labyrinthine deafness (two of these patients were merely under observation for this lesion). Only in 1 patient (a girl, 10 years old) with deafness refractory to ordinary treatment, no other clinical symptoms were noticed, but the serum reactions were strong and the mother and sibs had syphilis, so that the diagnosis congenital syphilis appears to be sure in this case too.

Symptom 8 comprises nervous symptoms. This group includes 28 patients, 10 males and 18 females. It may seem striking that the females are in the majority, but the difference is not significant. Here the age distribution is: 0—9 years, 5 patients; 10—19 years, 14 patients; 20—29 years, 3 patients; 30—39 years, 4 patients; ≥ 40 years, 5 patients. Of these patients 7 had dementia paralytica*) (2 males, 5 females); the youngest of these patients was 2 years, the oldest 23.

Among the remaining 21 cases mention is to be made of 3 instances of tabes (Nos. 24, 120, 170); 1 case of predominantly meningeal syphilis (No. 227); 4 cases in which the clinical features were most suggestive of endarteritic processes (Nos. 22, 121, 217, 307). The remaining 13 cases may be classified only as neurosyphilis; the youngest of these patients was a newborn, the oldest was 58 years.

In the case of this newborn patient (No. 307) it has to be considered whether the lesion may have been due to birth injury. The patient had clonic patellar and plantar reflexes, besides attacks of clonic convulsions lasting for hours. The mother had been under antisyphilitic treatment from the 6' month of pregnancy, and treatment of the child was instituted at the age of 3 weeks. The outcome was poor, however, as the child had to be admitted to an institution for mental defectives — at the age of 3—4 years.

In a female patient, aged 37, the symptoms consisted in sequelae from hemiplegia at the age of 1—2 years. In addition she presented chronic rhinitis and deformity of the teeth.

*) Two of these cases have been described before by Geo. K. Stürup in *Ugeskrift for Læger* 1939, p. 245.

One of the 13 patients, a boy, No. 282, aged 4 years, was possibly suffering from dementia paralytica with pronounced megalomania: associating daily with God, the king, Hitler and the devil, besides assaulting the other children. Spinal fluid: WR=5; cells 500/3; glob./alb. 1/35. Improvement on treatment.

Of the 28 patients with neurosyphilis 6 were not examined by lumbar puncture (Nos. 5, 16, 22, 24, 62, 96). In these cases the diagnosis was based on the clinical symptoms. In the 22 patients on whom lumbar puncture was performed, the results of WR, albumin/globulin determination and cell count on the spinal fluid were distributed as follows: 13 patients had + WR and showed pathological values for albumin/globulin and cell count; 3 patients had + WR, no other examination was made; 3 showed — WR but pathological values for albumin/globulin and cell count; and in 3 patients all tests on the spinal fluid turned out normal, whereas the clinical symptoms were unquestionable.

That 8 of the 27 patients were over 20 years old may seem a little strange; but the concomitant symptoms proved that the nervous symptoms were not attributable to acquired syphilis. 4 patients had parenchymatous keratitis, and 2 showed dental deformities, while 1 — a tabetic, aged 36 — had impairment of the hearing, and 1 — male, aged 23 (No. 74) — was paralytic. The patient with impairment of the hearing had previously had ulcers on the tonsils and palate and gave a family history of syphilis in the mother; the last-mentioned patient presented abnormal dentition, and 2 sibs had congenital syphilis, on which account the presence of scars on the glans are taken to be of no significance. In both of these cases there is a slight chance of acquired syphilis, but the lesion is judged rather to be of congenital origin.

Symptom 9 covers cases with Hutchinson teeth. (In some of the older patients the appearance of the teeth could not be estimated on account of caries or extraction). This is a large group, comprising 55 patients, although cases with »possible dental deformity« are excluded. It may seem strange that we have recorded dental deformity in 7 children, aged 2—6 years, but our information was obtained so long after the time of the diagnosis that four of these patients now were 7 years old, and the rest 8—9 years, so that it may have been possible to observe the appearance of the permanent teeth.

Symptom 10 includes other symptoms of specific nature. The group comprises 33 patients, 17 males and 16 females. The age distribution is as follows: under 1 year, 10 patients; 1—9 years, 10 patients; 10—19 years, 7 patients; ≥ 20 years, 6 patients. Naturally this group covers several symptoms of different character. The predominant symptom is universal adenitis, being recorded for 10 of the 22 patients who

were up to 10 years old, while 4 presented enlargement of the cervical lymph glands. 6 patients had choroiditis or chorioretinitis, while 3 patients had a heart lesion. Cardiac disease is no common symptom in congenital syphilis⁶⁾, and on a revision of the information first obtained, these cases turned out to be rather uncertain. It will be appropriate here to give the records of these three patients in abstract:

No. 17.

Woman, aged 42, who in October 1936 was admitted to the medical department of a hospital in Copenhagen for psoriasis, obesity, umbilical hernia and arterial hypertension; she gave a strong + WR. Congenital syphilis was diagnosed on the basis of the serum reaction and the family history of congenital syphilis in two sibs who died. X-ray examination showed enlargement of the heart shadow, but auscultation revealed no sign of any heart lesion.

No. 33.

Woman, aged 35. At the age of 12 years she had frontal ostitis and a joint lesion, said to be rheumatic fever; from the age of 16 she was suffering from a heart lesion. She was hospitalized from 12/7—25/7/38 for a urethral polyp and heart lesion. As she gave a strongly positive WR, congenital syphilis was diagnosed. Auscultation of the heart showed extension of the heart borders to 1 cm. laterally to the midclavicular line in the 5' interspace. The heart action was regular; a faint systolic murmur was heard everywhere; $P_2 = A_2$. After the discharge from the hospital, antisyphilitic treatment was instituted. She tolerated 2 bismuth injections and 0.15 cg. neosalvarsan very well; but after injection of 0.45 cg. neosalvarsan, serious cardiac phenomena appeared rapidly. The patient was readmitted to the hospital and died a few hours later. Auscultation of the heart: sounds faint, but without murmur. Electrocardiography, Leads II and III: large deflections; P wide and notched; T inverted. X-ray examination of the heart: no abnormality. Autopsy not performed. As cardiac disease due to congenital syphilis is observed but rarely, it seems more reasonable to assume that the joint and heart lesions in this patient may both be due to an attack of rheumatic fever. So the reason why the heart of this patient failed under the treatment remains obscure. In case of syphilitic heart lesion, treatment with salvarsan may be associated with sudden death. We should not venture, however, to take the death of the patient to support the assertion about a syphilitic heart lesion.

No. 51.

Woman, aged 27, with parenchymatous keratitis, headache, dizziness, and strongly positive serum reactions. At first the information was received that the patient presented an aortic murmur, but a later review of the case history disclosed that auscultation and X-ray examination of the heart showed no abnormality.

Thus the 3 heart patients*) are reduced to 1, and even this one is somewhat disputable.

*) This revision of the case histories with reference to the account of symptoms brings about that No. 17 becomes symptom-free, whereas No. 51 keeps the symptom formula 4, 10, as headache + dizziness in patients with a normal spinal fluid are recorded under the diverse symptoms (10).

In 6 cases Symptom 10 is recorded on the basis of data concerning a striking appearance of the patient: atrophic (2), small and delicate (1), thin and poorly (1), pale and flaccid (2). As we have not seen the patients ourselves, we are not able to state how many of them have really presented the typical features of »senile atrophy«. 5 of these patients were infants who also presented symptoms from the skin and mucous membranes; the 6th was a young man (No. 145) 16 years old, who sought medical advice for constipation; his appearance suggested the performance of a Wassermann test, which gave the diagnosis. Syphilis in the mother was diagnosed 2 years before the birth of the boy, and it was rediscovered a few months previously, on admission to hospital for other complaints.

Hydrocephalus was found in 3 of the patients with Symptom 10. Two of these patients are mentioned above (one with choroiditis, and one with a greyish pale flaccid appearance), and the third (No. 231) had keratitis and dental deformity.

The last 3 patients presented respectively syphilitic orchitis (No. 48) headache and dizziness which subsided on antisyphilitic treatment (No. 105) and severe anemia (No. 291). In these cases the diagnosis congenital syphilis is supported by information about congenital syphilis in sibs or unquestionable clinical symptoms.

*Symptom 12**) covers the mental development of the patient, with a special view to changes in their mental habitus. This group comprises 50 patients, 21 males and 29 females. The age distribution was as follows: < 1 year, 6 patients; 1—9 years, 10 patients; 10—19 years, 29 patients; 20—29 years, 3 patients; and over 30 years, 2 patients. It may seem peculiar that 6 patients are under 1 year of age; but we have received information about their mental development 2—5 years after the time when the diagnosis was made: 2 were imbecile, 1 was dull, and 3 were lagging in mental development, so that in this material no intelligence test was performed on any infant.

It is to be pointed out that no less than 18 of the patients with Symptom 12 presented also Symptom 8 (nervous symptom), but this is not to be wondered at as neurosyphilis in children is associated with a considerable mental damage.

The group of mental deficiency which in our material was represented by the greatest number of patients was the group »retarded mental development«, as no less than 22 of the patients were entered under this heading; then come the imbecile with 14 patients, and »childish behavior« with 4 patients; finally the following designations were each applied to 1—2 patients: mental defective, demented, stupid,

*) In our case-record forms, Symptom 11, symptoms and diagnoses which have nothing to do with syphilis; these data were so few in number that they are not taken into account here.

unbalanced, poor memory. As many of these children on account of their treatment for congenital syphilis have been in frequent contact with the physician (in some cases, through the Welander Home), the chance of discovering a minor deficiency in intelligence has been rather great, so that it would not be warrantable off-hand to establish that "mental deficiency" is a very frequent symptom among patients with congenital syphilis as compared to the total population. Finally, we have to consider whether the mental deficiency is due to the congenital syphilis, or whether the lack of interest in antisypilitic treatment on the part of the mother is due to inferior mental faculties, which turn up again in the child in the form of genuine mental deficiency together with the congenital syphilis.

Besides the above-mentioned patients with symptoms, a total of 84 did not show the least clinical sign of syphilis.

Of these patients 7 were from 20—35 years old (4 women and 3 men). In 4 of these cases the family history gave positive data of syphilis both in the mothers and in the sibs, while 1 patient (No. 73) gave information about maternal syphilis, and 2 (Nos. 39 and 88) had sibs with congenital syphilis. As a rule the serum reactions were strong (least in No. 52, with WR = 5, KR = 4).

In the 77 younger symptom-free patients a serum diagnosis was supported in 74 cases by positive data on maternal syphilis, and 1 patient had 2 sibs with congenital syphilis, whereas in the last 2 cases the diagnosis was entirely serological (Nos. 171 and 203, males, respectively 15 and 10 years old).

In the last 2 patients the chance of acquired syphilis was considered so slight that we did not hesitate in entering them in this group.

Summary.

An account is given of the collection of the material. The total material (comprising 406 patients) is presented and revised on the basis of additional information obtained.

Altogether 102 patients are ruled out from the present material; a fairly detailed mention is made of these cases.

Comparison is made with the account reported by Krag & Lindhardt for 1937—38.

A review is given of the material proper: 304 patients with newly-discovered congenital syphilis.

A review is given of the individual symptom groups (1—12), with a brief mention of the age distribution of the patients, the character of the symptoms and the certainty of the diagnosis, especially in those cases where the diagnosis is not so well founded.

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STUDIES ON CONGENITAL SYPHILIS DIAGNOSED IN THE PERIOD OF 1935—1939

II. COMPARISON WITH MATERIALS OF CONGENITAL SYPHILIS REPORTED PREVIOUSLY

By *Peter Krag and Esther Dalsgaard-Nielsen.*

The occurrence of congenital syphilis in Denmark during the last 50 years has been dealt with by Kissmeyer*) who plotted curves for the notified cases of congenital syphilis (absolute figures) in 1) the entire country, 2) Copenhagen, and 3) the rest of the country. These curves show three apices (in the late eighties, in the late nineties, and round 1920) and then a marked fall, from 236 to 72, in 1933. This fall is most pronounced for Copenhagen, where the figure for 1933 went down to 12. About 1921 the Copenhagen curve was crossed by the country curve, which from that time has kept at a considerably higher level than Copenhagen even though it has had a tendency to decline in the last years.

The age distribution of congenital syphilis is dealt with merely in the mention of the deaths, which nearly all took place in the first year of life. The geographical distribution is given for the period of 1924—29, as the relation between notified cases of congenital syphilis and acquired syphilis per county. Kissmeyer says himself that these figures are merely of orientating interest, as local conditions and accidental variations may give rise to some perplexing figures. As most of the cases of congenital syphilis in 1924—29 were diagnosed and notified after the first year of life (see Table 4 and Fig. 2), Kissmeyer should have limited himself to the incidence of congenital syphilis among infants,**) as this gives a more correct impression of the effectivity of the antisyphilitic treatment in the period concerned.

Even though our material is only half as large as that of Kiss-

*) Kissmeyer, A.: *Kønssygdommenes Optræden i Danmark i de sidste 50 Aar*. Ugeskrift for Læger, 97: 710, 1935.

**) After this paper was set in type we found that Kissmeyer in 1938 had given a survey of the frequency of congenital syphilis in the first year of life in relation to the number of births specially for Copenhagen. (Arne Kissmeyer: *The Combating of Venereal Diseases in Denmark*. Copenhagen. Fr. Bagge, 1938).

Table 2.
Frequency of Congenital Syphilis in the Individual Territories.

Territory	Per 5-year period.				
	Frequency of congen. syph. in patients < 1 year per 10,000 children born alive	Total number of children born alive in the period of 1935-39	All patients with congen. syph. No. of patients under 1 year in parenthesis	No. of families with congen. syph.	No. of patients with parenchymatous keratitis
Copenhagen and Copenhagen county	5.0	83,999	114 (42)	104	26
Rest of Sealand with surrounding islands	0.7	53,614	37 (4)	31	11
Fünen and surrounding islands	2.0	30,709	29 (6)	25	6
East Jutland	1.9	77,502	81 (15)	66	23
North and West Jutland	0.6	71,108	31 (4)	19	5
South Jutland	1.1	18,525	12 (2)	11	7
Total	2.2	335,457	304 (73)	256	78

meyer, we have investigated where in the country congenital syphilis was diagnosed (Fig. 1 and Table 2) and by whom the diagnosis was made (Table 3). It is to be emphasized that the material undoubtedly allows of a distribution merely on groups of medical districts (see Table 2), and that the fact that the physicians (in particular the specialists) mostly reside in the larger towns naturally brings about that patients residing in the country are put down for the nearest larger town, as we regret to state that we have no exact information about the residence of the respective patients. Still, patients who were diagnosed in Copenhagen but treated and kept under observation in a county medical district, are always recorded as belonging to this rural district.

Calculation of the frequencies for the individual medical districts would be of no interest, as the number of cases per age-class is small and influenced by accidental factors. Thus, for instance, the serological examination of all the patients admitted to a hospital may lead to the discovery of a syphilitic lot of sibs, and this will considerably increase the incidence of congenital syphilis in this district in one or more of the age-classes (see the groups of sibs on the map in Fig. 1).

The frequency of congenital syphilis per age-class and per 10,000

inhabitants is calculated for each of the territories*) mentioned in Table 2. These territories differ especially in the age-class of < 1 year,

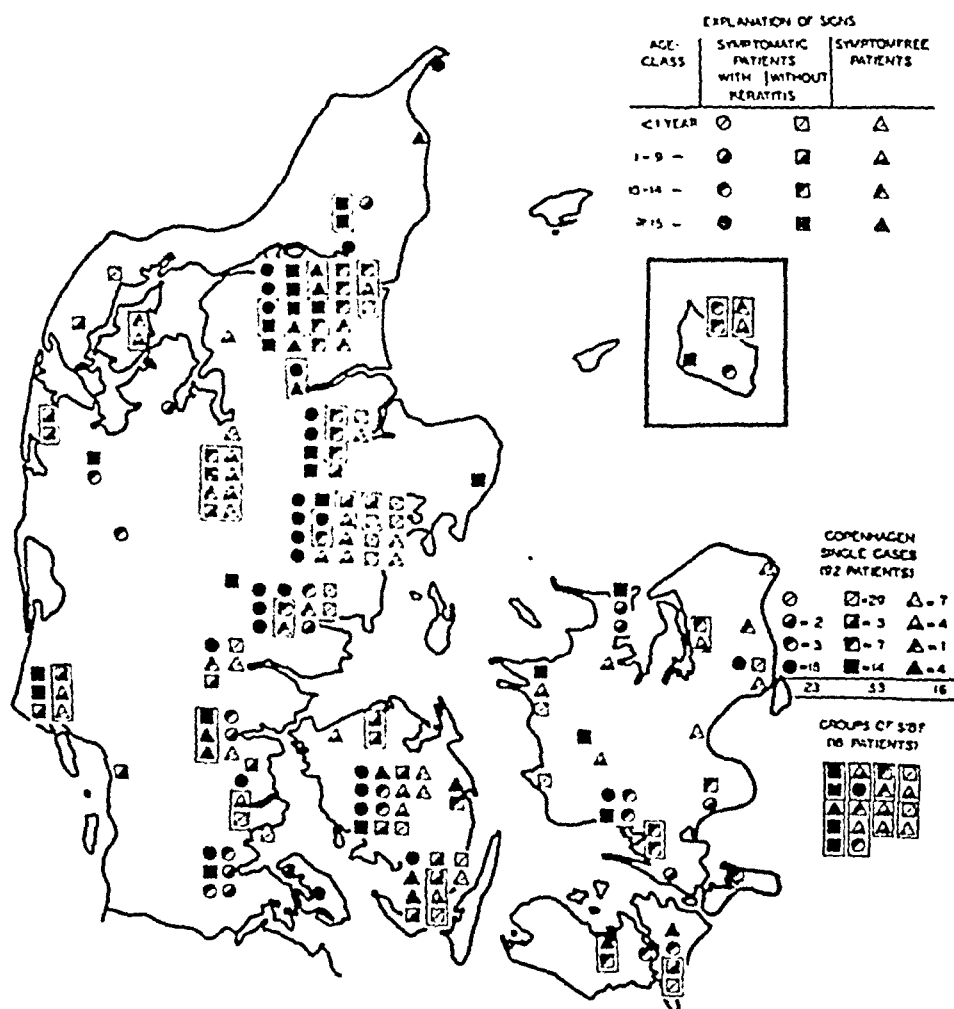


Fig. 4.

Map of Denmark showing distribution of 304 cases of congenital syphilis diagnosed in 1935-39.

All the cases outside Copenhagen are indicated by signs giving the age-class and a certain orientation about the symptoms. As far as space allows, the signs are placed at the residence of the diagnosing physicians. Signs for sibs are framed by a quadrangle.

In Copenhagen, 18 patients who make up 8 groups of sibs are indicated by signs and framing, while the remaining 92 patients are grouped in a table giving the number of patients per sign in the respective groups.

*) The figures of population are taken from Statistiske Meddelelser, series IV, vol. 105, No. 1, p. 36 and others.

The frequencies for the various age-classes and territories are not tabulated — only the analogous frequencies of the lesion in the first year of life per 10,000 births in Table 2.

Table 3.

Cases of Congenital Syphilis Distributed after Age and after the Character of the Diagnosing Physicians. Cases of Keratitis Recorded in Parenthesis.
304 cases from 1935-39.

Physician or hospital	Age- classes:	Copenhagen					Rest of the country					Entire country	
		<1	1-9	10-14	≥15	Total	<1	1-9	10-14	≥15	Total	Total	%
Obstetric and Pediatric Deps.		22				22	3				3	25	8.2
Derm.-ven. Dep and specialist		14	4	4(1)	4(2)	26(3)	4	2(1)	1	3	10(1)	36	11.8
Eye	" "		2(1)	1(1)	8(8)	11(10)	1	4(3)	5(5)	14(14)	24(22)	35	11.5
Psychiatric	" "												
Neurol.	" "		1	2	3(1)	6(1)			3	3(1)	6(1)	12	3.9
Medical	" "												
Surgical	" "	2	2	2	17(3)	23(3)	19	12(2)	8(1)	19(3)	58(6)	81	26.7
District Medical Officers							2	5	9(2)	5(1)	21(3)	22	7.2
General practitioners		1	5(1)	6(2)	9(4)	21(7)	5	28(5)	18(7)	21(8)	72(20)	93	30.6
Total		39	14(2)	15(4)	42(19)	110(25)	34	51(11)	44(15)	65(27)	194(53)	304	
% age distribution		35.5	12.7	13.6	38.2		17.5	26.3	22.7	33.5			
Frequency of all cases of congen. syph. and keratitis per 10,000 inhabitants for each age-class		7.24	0.31	0.53	0.12		1.33	0.22	0.32	0.06			
			0.04	0.14	0.06			0.05	0.11	0.03			

in which Copenhagen + Copenhagen county has 6.1 cases per 10,000 inhabitants, whereas Fünen and East Jutland have 2.1, South Jutland 1.1, rest of Sealand + adjacent islands 0.7, and North-western Jutland 0.6. For the age-classes of 1—9 and 10—14 years the values fall between 0.2 and 0.5 case per 10,000 inhabitants, the age-class of 1—9 years having most cases in Fünen (0.4), while the age-class of 10—14 years has its maximal values (0.5) in three territories: Sealand + islands, Copenhagen + Copenhagen county, and East Jutland. In the age-class over 15 years the two last-mentioned territories have 0.10 case per 10,000, while all the others show 0.03—0.07 per 10,000.

Here it is emphasized that congenital syphilis in newborn and infants constitutes a minor part of the material. This is seen plainly from the map of Denmark (Fig. 1) on which the cases are recorded after sex and age-classes < 1 , 1—9, 10—14 and ≥ 15 years. (The age-class of < 1 year corresponds to the blank symbols \square and \bigcirc .) In the lying-in hospitals in Aarhus and Copenhagen, respectively 3 and 15 cases were diagnosed, i. e., 25 % of all the cases under 1 year in the entire country.

In Table 3 an account is given of the kinds of physicians or hospitals who have made the diagnosis of congenital syphilis in the various age-classes in Copenhagen and in the rest of the country.

Congenital syphilis in infants was diagnosed in Copenhagen almost exclusively by obstetric, pediatric and venerologic departments, while in the rest of the country these departments largely are replaced by general hospitals or medical and surgical departments.

In the age-classes of 1—9 and 10—14 years the lesion was diagnosed in Copenhagen especially by venerological departments and practising physicians, while in the rest of the country the diagnosis was made by hospital physicians, district medical officers and general practitioners.

In the age-class ≥ 15 years, in Copenhagen as well as in the rest of the country, the diagnosis was made especially by eye departments and ophthalmologists, hospitals and general practitioners.

Considering the entire material under one, the diagnosis was made most often by general practitioners and by medical or surgical departments (respectively 31 % and 27 % of the cases). About 12 % of the cases were diagnosed by venerological departments and dermatologists and by eye clinics and ophthalmologists, whereas obstetrical and pediatric departments cover 8 %, district medical officers 7 %, and psychiatric departments 4 %.

So we should get a wrong picture of the occurrence of congenital syphilis (too few patients under 15 years) if we took the role played by the general practitioners in the diagnosis of the lesion to be insignificant and omitted to include these cases in the material.

The rather high frequency of congenital syphilis in the age-classes

of 1—9 and 10—14 years outside Copenhagen means hardly that congenital syphilis was particularly frequent there for up to 14 years ago, but rather that several latent cases were not diagnosed at first and were disclosed later by a routine examination or by the appearance of definite symptoms.

Of the total patients, 110 came from the city of Copenhagen, and this group shows a small, though not significant, preponderance of patients with definite symptoms (84 out of 110 as against 136 out of 194 in the rest of the country).

The diagnosis of keratitis — see Table 2, figures in parentheses — was made equally often by ophthalmologists and practising physicians; in some cases, however, this may be due to the circumstance that the patients are referred to an ophthalmologist, who then suggests that the patient's own physician should send the blood sample for the Wassermann test to the Serum Institute — so that the cost of the test will be covered by the health insurance.

The considerable accumulation in Copenhagen of children under one year with congenital syphilis is in keeping with the fact that the frequency of fresh syphilis in the period of 1935—39 was 3—4 times higher in Copenhagen than in the rest of the country.

As the lying-in departments of the Rigshospital admit unmarried parturients regardless of their residence, it is conceivable, however, that this brings about an extra accumulation of cases of congenital syphilis in the first year of life here in this town. We have been unable to get a survey of the residence of unmarried mothers in the year prior to the parturition and can only give the following data concerning this question; 14 out of the 39 children under one year in Copenhagen were born and diagnosed in the Rigshospital. The information received by the syphilitic card index shows that 12 of the 14 mothers were unmarried, and only two of these were under observation post partum by physicians outside Copenhagen (Køge and Farum). It cannot be learned where these 12 mothers resided at the time they contracted the infection, but it is most probable that the majority of them were living in Copenhagen.

In Copenhagen there is a non-significant accumulation of cases of congenital syphilis among the children from the lying-in hospitals but correlation of the 7759 children born in the hospitals of unmarried mothers (12 children with congenital syphilis) with all the other 41,613 births in Copenhagen (25 children with congenital syphilis) shows a statistically significant difference ($t=2.7$), cf. Section III p. 302.

It is further found that relatively few groups of sibs were diagnosed in Copenhagen, and as the members of these groups as a rule are of school age or adult, this will have a similar effect on the age distribution.

Table 4.
*Age Distribution for Various Materials of Congenital Syphilis — Total Materials,
Keratitis Materials and Specially Selected Groups and Periods.*

Origin	Material	Nature	Age groups*)				Total
			0—1	1—5	5—15	>15 years	
A1 Dalsgaard-Nielsen and Kissmeyer	Living congen. syph.		1215 (55.3)	257 (11.7)	564 (25.7)	160 (7.3)	2196
AII „ „	Living + dead congen. syph.		2027 (66.5)	274 (9.0)	580 (19.1)	165 (5.4)	3046
B „ „	Keratitis		3 (0.6)	72 (14.5)	310 (62.5)	111 (22.4)	496
C Dalsgaard-Nielsen (Havnegade clinic)	„		1 (0.2)	45 (10.8)	160 (38.6)	209 (50.4)	415
D Syphilitic card index registered 1935—39	All » congen. syph.«		105 (25.9)	40 (9.9)	97 (23.9)	164 (40.4)	406
E „	Excluded cases (Groups a ₂ —e)		32 (31.4)	5 (4.9)	8 (7.8)	57 (55.9)	102
F „	Material proper (Group a ₁)		73 (24.0)	35 (11.5)	89 (29.3)	107 (35.2)	304
G „	Material F symptom-free		22 (26.2)	18 (21.4)	24 (28.6)	20 (23.8)	84
H „	„ » symptomatic		51 (23.2)	17 (7.7)	65 (29.6)	87 (39.6)	220
I „	„ » without cicatricial cases		73 (24.6)	35 (11.8)	89 (30.0)	100 (33.7)	297
J „	„ H without cicatricial cases		51 (23.9)	17 (8.0)	65 (30.5)	80 (37.5)	213
K „	Keratitis, all cases		0	6 (7.7)	26 (33.3)	46 (59.0)	78
L „	„ sequelae		0	0	2 (12.5)	14 (87.5)	16
M „	„ acute		0	6 (9.7)	24 (38.7)	32 (51.6)	62

N ₁ Notified cases on											
Weekly Health Report											
2	1880-84	459	(87.6)	19	(3.6)	19	(3.6)	27	(5.2)	524	
3	1885-89	545	(89.4)	24	(3.9)	21	(3.4)	20	(3.3)	610	
4	1890-94	322	(85.0)	18	(4.8)	17	(4.5)	22	(5.8)	379	
5	1895-99	457	(82.6)	50	(9.0)	24	(4.3)	22	(4.0)	553	
6	1900-04	656	(89.7)	27	(3.7)	19	(2.6)	30	(4.1)	732	
7	1905-09	431	(84.5)	42	(8.2)	15	(2.9)	23	(4.5)	511	
8	1910-14	518	(72.9)	71	(10.0)	59	(8.3)	63	(8.9)	711	
9	1915-19	516	(58.6)	96	(10.9)	104	(11.8)	166	(18.8)	882	
10	1920-24	453	(50.7)	94	(10.5)	153	(17.1)	194	(21.7)	894	
11	1925-29	228	(36.6)	87	(13.9)	141	(22.6)	168	(26.9)	624	
12	1930-34	107	(22.8)	44	(9.4)	148	(31.5)	170	(36.2)	469	
13	1935-39	62	(22.1)	32	(11.4)	70	(25.0)	116	(41.4)	280	
O	From Material A	934	(85.5)	68	(6.2)	79	(7.2)	11	(1.0)	1092	
P	"	651	(69.5)	74	(7.9)	147	(15.7)	64	(6.8)	936	
Q	"	349	(44.3)	113	(14.4)	264	(33.6)	61	(7.8)	787	
R	"	93	(40.2)	19	(8.2)	90	(39.0)	29	(12.6)	231	
S	Notified cases on										
Weekly Health Report		1365	(69.0)	194	(9.8)	174	(8.8)	246	(12.4)	1979	
T	"	594	(46.4)	147	(11.5)	242	(18.9)	297	(23.2)	1280	
U	From Material B										
V	"	2	(1.1)	3	(15.0)	14	(70.0)	3	(15.0)	20	
W	"	1	(0.4)	19	(10.3)	112	(60.8)	51	(27.7)	184	
X	"			44	(19.7)	139	(62.3)	39	(17.5)	223	
	"			6	(8.7)	45	(65.2)	18	(26.1)	69	

*) Figures in parenthesis give the percental age distribution for the line concerned.

As mentioned in the introduction of the preceding paper, one of the reasons for taking up this work was the great difference between the age distributions of the two materials of keratitis reported in Dalsgaard-Nielsen's dissertation*) (see p. 73—85). The part of the material that came from the eye clinic in Havnegade included more older patients than the part originating from the card index of the late Professor Kissmeyer. The age distribution is shown in Table 4, lines B and C, and it may be mentioned that the age groups over 15 years amounted respectively to 50 % and 22 % of the cases.

The difference in age distribution is very great and statistically significant, as the probability of this difference being accidental is exceedingly slight ($P < 0.000001$ %).

As our 62 patients with new-diagnosed acute and subacute keratitis showed an age distribution (Table 4, line M), which quite corresponded to the age distribution of the Havnegade material (52 % of the patients over 15 years), the composition of the different materials had to be submitted to a detailed analysis.

Dalsgaard-Nielsen's total material of congenital syphilis from the period of 1860 to 1930 (Table 4, line A_I) cannot be estimated under one, as the age distribution of the new-diagnosed cases naturally changes through this period, corresponding to the changes in treatment and diagnostic adjuvants. Besides, the number of patients who died, altogether 850 (see line A_{II}), who were excluded by Dalsgaard-Nielsen, have to be included too in order to obtain the total material comparable with other materials of congenital syphilis (761 out of the 850 died in the first year of life).

On investigation of a fairly long period with increasing effectivity of the antisyphilitic treatment of the mothers and decreasing frequency of fresh acquired syphilis, the yearly number of new-diagnosed cases of congenital syphilis should decrease; in the first years, however, this fall will manifest itself only among the newborn, later also among infants and school children, whereas it will take over 20 years before the figures for new-diagnosed cases of congenital syphilis in adults are influenced by the factors mentioned.

Accentuation of the sensitiveness of the serum reaction will increase the number of symptom-free cases, as patients who previously showed weak or transitory reactions now with the more sensitive methods give positive reactions lying several degrees over the zero point, implying the possibility of a sure serum diagnosis. But this is not likely to influence the age distribution to any particular extent — cf. the age distribution in Table 4, lines G and H (symptom-free and symptomatic) — as the few symptom-free patients over 15 years, indeed, are an expression of our caution.

*) Keratitis parenchymatosa luica og dens Folgetilstande særlig belyst gennem sene Efterundersøgelser. Copenhagen, 1938.

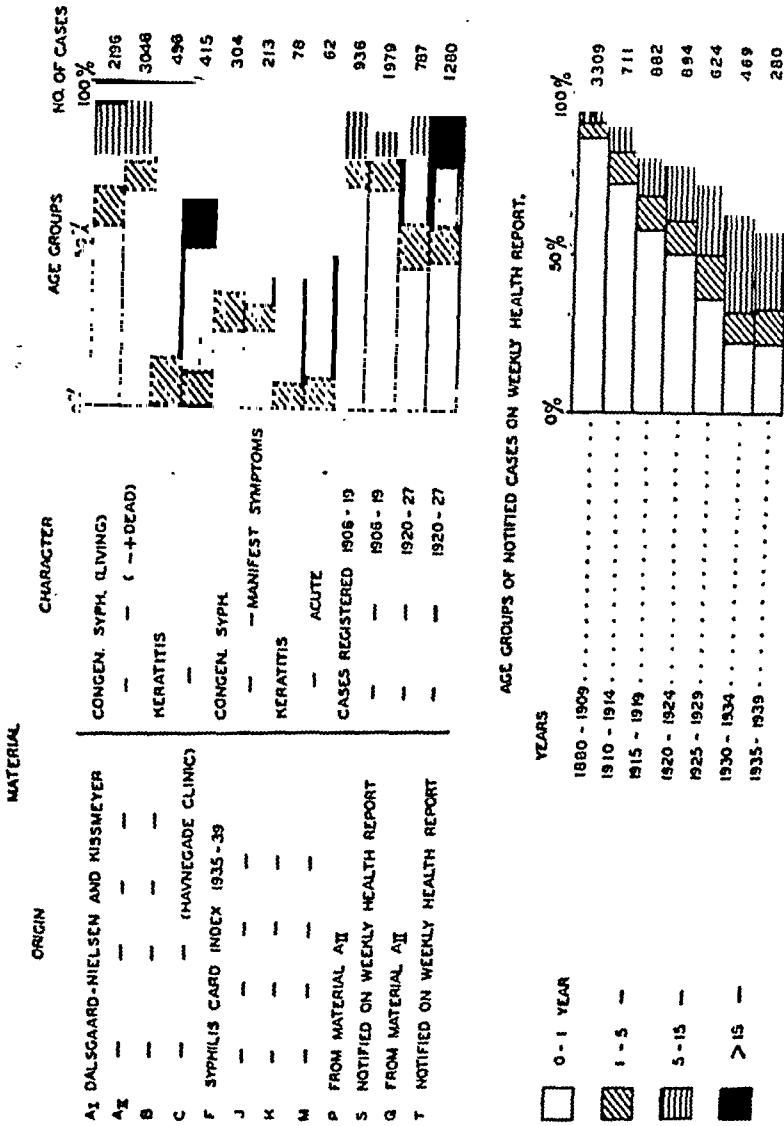


Fig. 2.

Graphical presentation of various materials of congenital syphilis — total materials, keratitis materials and especially selected groups and periods.

The increased routine employment of the serum reactions promotes the discovery of cases free from symptoms or cases with syphilitic symptoms which do not make the patient seek medical advice. Perhaps this increases in particular the number of older cases of congenital syphilis. On the other hand, the frequently accidental diagnosis of latent acquired syphilis in the mothers will bring about that latent congenital syphilis in *younger children* will be discovered, whereas familial examination of older children who are no longer living at home often has to be given up. Hospitals and practising physicians will often hesitate to start a familial examination when latent syphilis, for instance, is diagnosed in a woman, 65 years old, with 5 adult children who are claimed to keep well.

A certain possibility for estimation of the age distribution of new-diagnosed cases of congenital syphilis through a given period is found in the weekly lists of notified cases of congenital syphilis. Krag & Lindhardt*) have demonstrated that in the years of 1937—38, 1940—41 and 1942 the notifications of cases of congenital syphilis were encumbered with rather great errors — they were too few, and some of them were even false — but the age distribution was correct; and of our material from 1935—39 (line F or I) it also holds true that the age distribution is about the same as for the total figure of the National Health Reports for this period (see Table 4, line N 12).

The difference between the age distributions in N 12 and in I (> 15 years, 41 % as against 34 %) is slight, and the probability of this distribution being accidental is 7 %.

From 1880 to 1939 the figures of the National Health Reports (Table 4, lines N 1—12 and Fig. 2) are divided into 5-year periods. From 1880 to 1909 about 85 % of the notified cases were patients under 1 year, and the remaining 15 % were distributed equally over the age-classes 1—5, 5—15 and > 15 years. In the period of 1910—34 there was a gradual transition to the present age distribution, as for each 5-year period about 10 % of the cases were transferred from the class of infants to older age-classes:

1905—09:	< 1 year,	84.5 %;	> 15 years,	4.5 %
1930—34:	"	22.8 %;	"	36.2 %.

The absolute figures are found to be fairly uniform for infants till 1920—24, whereafter there is a rapid fall from about 100 per year to about 12 per year.

The older age-classes keep constant (at a low level) till 1910, reach a maximum (about 35 cases per year) in the period of 1915—34, and then fall off again.

So the distribution is quite in keeping with what was to be expected after the introduction of the salvarsan therapy and the Wassermann test.

*) Ugeskrift for Læger, 101: 1495, 1939 and — — — 1944 not yet appeared.

It would not be justifiable, however, from these figures to conclude that in the first-mentioned period congenital syphilis actually was a rare lesion in older children and adults; undoubtedly this was due to the circumstance that the diagnosis could be made only with difficulty prior to the appearance of the diagnostic adjuvant: the serum reaction.

For theoretical reasons one may regret that the salvarsan therapy and the Wassermann test appeared at such a short interval; a period of 15—20 years with mercury therapy and Wassermann control would have been of interest.

For appraisal of Dalsgaard-Nielsen's materials of congenital syphilis and of keratitis we will employ a division after the time of the diagnosis (= Dalsgaard-Nielsen's time of registration), dividing each material in the following four periods: until 1906, 1906—19, 1920—27, and after 1927.

As to *congenital syphilis*, see Table 4, lines O, P, Q and R, where the age distribution for the four periods is given. It will be noticed that also Dalsgaard-Nielsen's material shows the same change in type through the years as do the notifications: prior to 1906, 85 % < 1 year; after 1927, 40 % < 1 year. For the two intermediate periods it was possible for Kissmeyer and thus also for Dalsgaard-Nielsen through the syphilitic card index to pick out cases of congenital syphilis. For the period of 1906—19, however, this was practicable only when the physicians on the accompanying note provided information about the patient's lesion. For the period of 1920—27 this possibility was also furthered by the increasing serological diagnosis or verification of the clinical diagnosis of congenital syphilis, which directly entered the cases in the syphilitic card index. We therefore have compared these two sections, which correspond to the best part of Dalsgaard-Nielsen's material, with the cases of congenital syphilis notified in the same period (Dalsgaard-Nielsen's material on lines P and Q; notifications on lines S and T). Thus there are found to be at least twice as many notifications as cases in Dalsgaard-Nielsen's material. In addition, the age distribution shows that in Dalsgaard-Nielsen's material a good many cases were wanting in the age-class of > 15 years. The difference between the two materials is so pronounced that a calculation of the probability of the difference being accidental gives $P < 0.00001$ %.

Of the 580 dead patients now included (see above), in periods II and III, respectively 235 and 131 were under one year, 10 and 16 over one year.

Investigation of Dalsgaard-Nielsen's *keratitis material* (line B) — the division of which in the 4 periods mentioned is given on lines U, V, W and X — shows the surprising feature that the age distribution

practically is the same in four periods (V and W differ a little, but this corresponds merely to a probability of $P = 1.5 \%$, and presumably this is due to the circumstance that Kissmeyer from the hospitals and clinics obtained particularly thorough information about keratitic patients for the years prior to 1920, so that the older patients were more fully represented in this period than after 1920).

As the age distribution is the same for Havnegade patients from 1890—1912 and for our material from 1935—39 and lies at a level considerably different from that of all Dalsgaard-Nielsen's four periods, we have to conclude that Dalsgaard-Nielsen's material is lacking a considerable part of the cases of keratitis diagnosed in patients over 15 years (the difference between that one of Dalsgaard-Nielsen's periods which has relatively most patients over 15 years and our material is so great that the calculation of probability gives $P =$ about 0.1%).

A particularly critical analysis has been applied to the case records of 32 patients over 15 years in our material with this outcome: In 13 cases the diagnosis was supported by information of maternal syphilis (and possibly syphilis in the sibs too). In 4 cases the diagnosis was corroborated by other clinical symptoms, and in 6 cases by other clinical symptoms as well as data in the family history.

In the remaining 9 cases only the presence of keratitis and a positive Wassermann test were disclosed, but the age of these patients, 16—39 years, together with the year of the diagnosis suggested these patients may hardly have had an acquired syphilis of earlier date, as this would most likely have been recorded in the syphilis card index since these patients in 1920 were only from 1 to 22 years old.

From the literature it is evident that acquired syphilis gives parenchymatous keratitis but seldom and as a rule only in patients presenting other signs of secondary eruption — on which account it is to be emphasized that our above-mentioned 9 patients presented no evidence of acquired syphilis according to the information received from the respective physicians.

In the conclusion of this work efforts were made to establish decisively whether these 32 patients really were suffering from acute keratitis — through a review of the copies of the case records and a number of the letters from the respective physicians — with the following outcome:

In this way the presence of acute keratitis was verified in 27 cases, while now (in 1942) 3 patients could no longer be found by the physicians who three years ago declared the lesion to be parenchymatous keratitis; and new data were obtained concerning 2 patients. In one of the latter cases — a patient of 18 years — the original information accompanying the blood sample for the Wassermann test said that keratitis and hyarthrosis of the knee were present, whereas now we received the answer that the case record in the hospital merely has the note »knee lesion in the patient, but eye lesion in the sister of the patient«. A few weeks later the treating

physician stated in the case record: »Bad knees and keratitis, but no detailed data.« So this case is not quite cleared up, as the patients may have had either remnants of an old keratitis or a recent keratitis. — The other patient is 19 years old and suffering from iridocyclitis (and dental deformity), but this affection has to be considered equivalent to keratitis (cf Section I, p. 274).

This means that the information obtained about 29 patients shows that 28 of them were suffering from acute keratitis. Concerning the remaining 3 patients — respectively 19, 18 and 16 years old — no additional information could be obtained; but, like all the other 29 patients, their eye lesion was diagnosed by ophthalmologists, so that it is most likely that they too were suffering from acute keratitis.

As the Kissmeyer materials are based on data in the Wassermann card index, these differences are rather striking. It will be appropriate, therefore, in detail to mention some aspects of the Wassermann card index and its employment.

From Dalsgaard-Nielsen's dissertation (*l. c.* p. 13, ff.) we know the technique adopted by Kissmeyer for the collection of his materials:

- A. The basal material originates from the Wassermann card index of 1906—27 (years of birth); here all the cards were picked out that were marked as congenital syphilis and, in addition, the cards on seropositive patients not over 16 years.
- B. The material was then amplified in various ways:
 1. Review of case records in the Rudolph Bergh's Hospital and in Dep. H. of the Rigshospital covering the same period.
 2. Archive of Dep. IV of the Kommune Hospital covering congenital syphilis from 1860.*)
 3. Cases of congenital syphilis with which Kissmeyer became acquainted in other (more accidental) ways.
 4. For the period of 1927—32 the case records in the above-mentioned three hospitals constitute the basal material.

In this way Kissmeyer obtained a large but heterogenous material, from which Dalsgaard-Nielsen picked out the 2196 patients who, according to the data in the card index, were still living — and this procedure was quite justified as the original purpose was merely to obtain the collection of patients suitable for follow-up studies.

Whether the total material proper (Table 4, line A_{II}, 3046 cases) may be taken as a true expression of the registration of congenital syphilis will be evident from the following, as we shall investigate whether it was practicable to obtain the serviceable material from the Wassermann card index by picking out the cards after the Kissmeyer method or after any other method.

Prior to 1934 only the serum reactions (and the necessary in-

*) Perusal of the Kissmeyer card index has shown that many of these children died shortly after syphilis had been diagnosed — serious eruptions on the skin and mucous membranes.

formation about the data and the physician) were registered in the Wassermann card index, whereas anamnestic and clinical data were not requested systematically.

From 1935 one or more questionnaires concerning the anamnestic and clinical aspects are sent to the physician in every new-diagnosed case regardless of the age of the patient, and the obtained data are registered. For every month, then, a list is made of all new-diagnosed cases and the data obtained. Then the cases are classified by means of a figure code, making it easy at any time to pick out clinically and anamnastically uniform series of cases diagnosed in one of these later years. Our present material was obtained in this way. For the sake of illustration it may be mentioned that it *may* be impossible from an old Wassermann index card — for instance, for a man, 25 years old, who has registered in 1922 on account of a positive reaction — to see whether he was suffering from a recently acquired syphilis, syphilis diagnosed and poorly treated abroad since 1920, new-diagnosed congenital syphilis, or congenital syphilis diagnosed and treated 20 years previously.

Owing to the organization of the card index at that time, Kissmeyer has not been able to pick out patients over 16 years unless the physician of the respective patient of his own accord had given some data concerning the possibility of congenital syphilis. This naturally gives rise to a certain skewness of the material which was remedied to some extent, but far from sufficiently by the additions mentioned under B 1—3 (p. 294).

So it has not been practicable at all prior to 1935 from the syphilitic card index to obtain a material of congenital syphilis with a harmonic representation of all age-classes.

We have thoroughly gone through some parts of the annual material (years of birth) for 1907, 1912, 1917, 1922 and 1927 in the Kissmeyer card index and the Wassermann card index, and we have been able in this way to verify the statements made about the technique in picking out the cases for a given purpose.

In her dissertation, Dalsgaard-Nielsen (*l. c.* p. 22—23) demonstrated a deficit of 549 cases under 14 years in the Kissmeyer card index as compared to the Wassermann card index for the period of 1920—30; and we have further analyzed this deficit for the years of 1922 and 1927. It was found that the cases omitted chiefly represented either patients who were examined only once, regardless of the serum reaction*), or patients whose first serum reaction was performed after 1928, when Kissmeyer gave up the Wassermann

*) We have reviewed some of the case records of these card index patients with only one examination and found that Kissmeyer had to leave out these cases. For some of these cards corresponded to cases that could not be verified by the physicians (hospitals) who sent the blood samples to the institute, while others possibly may have resulted from failure of the registration system (specimens received under wrong dates, without reference to previous tests).

card index and contented himself with the case records in the hospitals of Copenhagen as a base for his material.

That the Wassermann card index in addition may include a few well-examined patients who apparently ought to have been included in the Kissmeyer card index is presumably to be taken as an expression of an accidental error; but this plays no numerical role.

Accordingly, the Kissmeyer material employed by Dalsgaard-Nielsen constitutes no homogeneous entity; and unfortunately the addition of the Havnegade material and other older patients gave the material an appearance of completeness.

Dalsgaard-Nielsen used her materiel to trace patients suitable for follow-up studies. On account of the starting material the 75 re-examined patients from the Kissmeyer material were relatively younger (age distribution: 23 % over 15 years) than the 49 cases from the Havnegade material (30 % over 15 years and the 49 cases Dalsgaard-Nielsen had collected herself (30 % over 15 years). It probably makes no particular difference that the age-class of > 15 years comprises a little fewer reexamined patients than it ought to include, since the analysis of the reexamined has taken into account the age of the patients at the time of the diagnosis.

The material given by Dalsgaard-Nielsen in her dissertation in Tables 14, 15 and following is *merely* a survey of the cases employed by her, and the readers are warned not to assume that it involves a general survey of congenital syphilis or keratitis through all the years given. In view of the defects in the material now demonstrated, it has to be looked upon as an askew section of the total cases diagnosed in the given period. But it has to be emphasized that owing to the organization of the syphilitic card index at that time, Kissmeyer had no possibility of obtaining a better material.

Dalsgaard-Nielsen and Dr. Rasch*) considered whether the great differences between the Havnegade material and the Kissmeyer material might be attributable to migration of patients to and from Copenhagen, a change in the age for the appearance of keratitis, or changes in the intensity of registration. But they did not arrive at any conclusion decisive enough for publication.

Summary.

The geographical distribution of the lesion is reviewed; a considerable part of the newborn with congenital syphilis come from the lying-in hospitals of Copenhagen and Aarhus.

The frequency of the lesion per county depends, among other factors, on the frequency with which the Wassermann test is employed by the hospitals as a routine measure of examination.

*) Dalsgaard-Nielsen, dissertation, l. c. p. 86—96).

Also the distribution of the material on various categories of physicians and hospital departments is investigated.

The age distribution for materials of congenital syphilis and keratitis is reviewed.

The material (in Dalsgaard-Nielsen's dissertation) originating from the syphilitic card index have a younger age distribution than the clinical material (Havnegade), the notified cases and the recent card index material, as the Kissmeyer materials have too few patients over 15 years.

The possibility of picking out the harmonic material from that part of the syphilis card index which was elaborated in 1920—34 is investigated; and it is pointed out that such a material obtained in that period naturally will include relatively too few patients over 15 years.

STUDIES ON CONGENITAL SYPHILIS DIAGNOSED IN THE PERIOD OF 1935—1939

III. CORRELATION OF SYMPTOMS IN CONGENITAL SYPHILIS

By *Peter Krag* and *Esther Dalsgaard-Nielsen*.

In the following an account will be given of the interaction of symptoms of the lesion as recorded in the present material. In addition we shall investigate whether the frequency of the well-known symptom complexes — dyads and triads — is great enough to be of any practical significance in the modern Danish material.

In this investigation, we think, it is of interest only to correlate the symptoms found in the patients at the very point of time when the diagnosis was made. Hence, in the following we shall leave out of consideration a number of symptoms, namely:

1. Symptoms which at the time of the diagnosis had to be interpreted as scars after a previous acute manifestation of the lesion or merely as corresponding to the data in the past history of the patient.

5 patients presented scars after skin symptoms; 16 gave a history of keratitis some or many years previously; 8 had previously had joint complaints; and, finally, in one case the following symptoms were recorded: past chorioretinitis, healed frontal otitis and hemiparalysis after a brain lesion in childhood. (Dental deformity and saddle-nose are never reckoned as »scar-like« phenomena, as they are never found in an acute reparable form.)

2. Symptoms that did not appear until after the time of the diagnosis: 7 cases of dental deformity (Symptom 9) observed at the age of 7—8 years in children whose congenital syphilis was diagnosed at the age of 2—6 years; and 5 cases of mental deficiency (Symptom 12) which was noticed subsequently in children whose syphilis was diagnosed before the age of 6 months.
3. The symptom of inferior mental development (Symptom 12) is excluded in the cases of 18 patients who at the same time presented signs of cerebrospinal syphilis (Symptom 8), as the answers re-

ceived on our questionnaire do not allow us to decide whether the lowered mental development was due entirely to the brain lesion or whether it involved a mental deficiency which later was complicated by cerebrospinal syphilis whereafter the disease of the patient was diagnosed.

A close study of the case records ought to have been carried out with a view to the point of time for the appearance of each of the many symptoms, as this constitutes the essential foundation for a study of the age of manifestation for the individual symptoms. — When a new material for the period of 1940—44 is collected, the study of the age aspects and the correlation of symptoms will be taken up again.

It was found reasonable to divide the studies on the symptom correlation into two main sections.

- I. One group comprising 44 patients at the age of 0—2 years with eruptions on the skin and/or mucous membranes, together with 3 infants with syphilis of the liver and 2 infants with syphilis of bones — altogether 49 cases. Probably all these patients had an acute infection with spirochetes at the time of the diagnosis.
- II. The other group comprises all the remaining symptomatic patients aged from 0—58 years, regardless of the character and number of symptoms — altogether 160 patients.

If the entire material were treated under one, we should find that eruptions on the skin and mucous membranes rarely coincide, for instance, with keratitis. The demonstration of this would not be of any particular interest, as it is due entirely to the great difference in the age for the manifestation of these symptoms.

The above mentioned exclusion of symptoms gives a decrease in symptoms per patient. In Table 5 a survey is given of the frequency of the various numbers of symptoms. It may be added that the symp-

Table 5.
Frequency of Patients with 1, 2 ... 5 Symptoms.

	Before revision of the material		After revision of the material	
	No.	%	No.	%
1 symptom	119	(54.1)	108	(51.7)
2 symptoms	58	(26.4)	63	(30.1)
3 »	30	(13.6)	32	(15.3)
4 »	9	(4.1)	6	(2.9)
5 »	4	(1.8)	0	
Total patients	220	(100.0)	209	(100.0)

Table 6.

Survey of Relation between Age-classes and the Individual Symptoms besides the Number of Symptoms per Patient.
Group I (Manifest Spirochætal Infection in Infants is recorded separately).

Age groups	Symptoms designated by code												No. of patients with No. of symptoms			
	1*)	2*)	3*)	4	5	6	7	8	9	10	12					
Group I	0-2	Years	39	27	16						10	49	11	25	10	3
Group II	0-4	„	1									10	8	1	1	
	5-9	„		2				2				23	11	9	3	
	10-14	„	1	4			4	7	23	3	11	47	26	11	9	1
	15-19	„		1	1		1	7	11	3	7	39	26	7	4	2
	20-24	„	1	1			2	3	2	1	2	17	12	3	2	
	25-29	„	1							2	2		8	6	2	
Entire Group II	30-34	„						1	2			5	4	1		
	35-	„		3			5	4	2	3		11	4	4	3	
			4	11	1	62	16	20	13	27	48	22	97	38	22	3
Code A																
Percent	0-9	„		19												
age distribution	10-24	„		56												
for Group II	25-			25												

*) As to Symptoms 1, 2 and 3, it is to be mentioned that in Group I they are very often combined: 38 patients presented at least two of these three symptoms. Group II includes no patients with a combination of these three symptoms; on this account, in some tables it has been convenient to record them under the joint designation Code A.

toms recorded for the 11 patients who on revision were found to be symptom-free, were as follows: keratitis (4 cases), joint lesions (2), keratitis + joint complaint (1), dental deformity (2), and inferior mental development (2).

An orientating survey of the distribution of symptoms on the age-classes for the 209 patients is given in Table 6, which also gives information about the frequency per age-class of patients with 1, 2, 3 and 4 symptoms.

The age distribution for Group II shows that 64 % of the patients belong to the age-class of 10—24 years, whereas the younger and older age-classes only comprise respectively 21 % and 15 % of the material.

A similar grouping is found for several of the individual symptoms (see Table 6, the left part): keratitis (Symptom 4), bone lesions (Symptom 5), affection of the central nervous system (Symptom 8), and deformity of the teeth (Symptom 9). — On the other hand, patients with joint lesions (Symptom 6), diverse symptoms (10) and mental deficiency (Symptom 12) are on an average somewhat younger (Symptoms 6 and 12 were present in 5 % and 0 % over 25 years; Symptom 10 was present in 45 % of the patients in the age group 0—9 years).

For Symptom A (lesions of the skin, mucous membranes and liver) the number of patients in the age-class 10—24 years is a little lower, but the distribution does not deviate essentially from that of the total material. Patients with ear lesion (Symptom 7) are encountered only in the age-class of 10—24 years (54 %) and over the age of 25 years (46 %), so that they differ greatly from the rest of the material.

A more detailed account of the age aspects of the individual symptoms is impracticable in this material as the exact age at the time of the appearance of the individual symptoms is not known. Often it was certain symptoms — *e. g.*, keratitis — that brought the patient to the physician, on which occasion other concomitant symptoms were discovered — and in the present material, unfortunately, these symptoms are all recorded for the age of the patient at the time of the appearance of the keratitis even though they might have preceded the keratitis perhaps by a considerable length of time.

The frequency of patients with one symptom varies from one age-class to another, and it appears to be increasing within the range of 5—35 years — but this may be due, among other things, to the afore-mentioned exclusion of symptoms, as in particular several of the older patients had »scar-like« symptoms, which had not led to the diagnosis of congenital syphilis at the time when these symptoms were acute.

Here the coincidence of the symptoms will be investigated only for the 49 patients in Group I and for the age-class of 10—24 years

in Group II, as from Table 6 we know that all the symptoms were present in this part of the material to a fairly plentiful extent.

In the large correlation tables (Tables 7 and 8) we have recorded all the observed coincidences of the individual symptoms. For instance, a patient with Symptoms 4, 5, 9 and 12 is recorded in the squares 4—5, 4—9, 4—12, 5—9, 5—12 and 9—12, altogether in 6 places, *i. e.* each of the symptoms of this patient will appear in 3 of the squares of the table.

From Table 8 we are able to read directly, for instance, that 10 patients presented the combination of keratitis (Symptom 4) and dental deformity (Symptom 9).

In order to assess the frequency of this combination, we have to take into account the total frequency of the two symptoms: Among the 103 patients with symptoms, 42 had keratitis, while 36 presented dental deformity. On this basis the following survey can now be set up;

	Keratitis		Total
	present	absent	
dental deformity			
present	10	26	36
absent	32	35	67
Total	42	61	103

From this the probability of the combination being accidental can be calculated, as the marginal figures are taken for granted. If the lowest value expected*) is ≥ 5 , it will be enough to calculate a *t* value for the frequency 10/36 and 32/67, which is done most easily by means of a nomogram given by Hald & Rasch (see, for instance, Friedberg's Dissertation).**)

If the smallest value expected is < 5 , a calculation of a binomial distribution***) is employed, the probability of the term observed being calculated first; then an estimate is made of the sum of the probabilities for the more extreme terms possible.

In the above mentioned case the expected value (14.7) is greater than the observed. The *t* value from the nomogram is 1.85 — which corresponds to the probability $P = 6.4\%$ for the material being a random sample. Such a coincidence in ten cases is no rarity.

As an example of frequent coincidence of symptoms, it may be mentioned that the combination of bone lesion and dental deformity (Symptoms 5 and 9) is encountered 7 times, while the expected value

*) Here the »expected value« for the coincidence of keratitis and dental deformity is $\frac{32 \times 36}{103} = 11.4$ *i. e.* somewhat higher than the value observed.

**) R. Friedberg: Studier over ikke-hæmolytiske Streptokokker. Dissert. Copenhagen 1941.

***) R. A. Fisher: Statistical Methods 6' ed., Chapter 21, 02, 1936.

M. Bjerrum & Th. Busk: Ugeskrift for Læger: 105, 826, 1943.

for their coincidence is only 3.5, as the symptoms appear altogether 10 and 36 times respectively. The calculation of binomials gives a probability of merely $P = 3\%$ for the observed number being a random sample.

The monosymptomatic cases are found along the oblique line of the table (recorded as coincidence with the symptom itself). The probability of these cases is calculated in a similar way as above, for instance, correlating the frequency of Symptom A and the frequency of monosymptomatism in a tabulary form as the following:

	Monosymptomatism	Plurisymptomatism	Total
Symptom A			
present	1	8	9
absent	63	31	94
Total	64	39	103

(here the expected value is 5.6. The binomial calculation gives $P = 0.16\%$, *i. e.* there are fewer cases with Symptom A alone than expected, and the deviation is significant).

In Tables 7 and 8 the results of the probability calculation are indicated by means of the designations (S), S, SS, and SSS, which signify the probability of 7—5 %, 5—1 %, 1 %—1 ‰ and $< 1\text{‰}$ respectively, while the expected values deviating more than 2 from the observed values are recorded in brackets.

Group I (Table 7) show no significant accumulation of coincidences of symptoms. On the other hand, certain constellations are encountered less frequently than expected, namely: lesions of the skin and liver, lesions of the mucous membranes and bones; the same refers to lesions of the skin and of the mucous membranes which, when alone, occur less frequently than expected — and these two deviations are decidedly significant. The most frequent constellation — lesions of the skin + mucous membranes — is seen 22 times, and this corresponds to the expected value.

Tabulating the material with symptoms from the skin, mucous membranes and liver grouped together as Symptom A, we find expected values everywhere except for »bone lesions« and »diverse symptoms«. These two symptoms occur as sole symptoms 2 and 0 times respectively. This tendency to plurisymptomatism is highly significant.

Finally mention is to be made of the 7 symptom formulae in the 13 patients who presented 3 or 4 symptoms (see Table 9). Only the formulae 1 + 2 + 3 and 1 + 2 + 10 are found 3 times; the others are observed once or twice. If Symptoms 1, 2 and 3 are grouped together as Symptom A, 4 patients present the formula A + 5 + 10.

The 103 patients of Group II present a highly variegated picture (see Table 8). Here 9 symptoms make their appearance, but many cells include only 1—2 cases. The most frequent combination is 4 + 9 (*cf.* the calculation above), but as the value expected is 14.7, no significant relative antagonism is involved ($P = 6.4$).

Table 7.

Survey of the Coincidence of the Symptoms in the 49 Patients of Group I (infants) and the Chance of obtaining these Constellations accidentally.

1	2	3	5	10	Symptom	Code	No. of patients
5 SS (8.8)	22	10 (S) (12.7)	8	8	Skin eruption	1	39
	1 SSS (6.1)	6 (8.8)	3 S (6.1)	5	Mucous membranes involved	2	27
		3	3	4	Liver lesion	3	16
			2	4	Bone lesion	5	11
				0 (2.2)	Diverse sympt.*)	10	10
	A	5	10		Symptom	Code	No. of patients
	32	9	10		Skin — mucous membrane — liver lesions	A	47
		2 SSS (7.6)	4		Bone lesion	5	11
			0 SSS (6.9)		Diverse sympt.	10	10

The figures give the number of cases in which the coincidence of the symptoms was observed. The expected number of such cases is recorded in parenthesis if it differs from the respective observed value by more than 2. The *S* signs give the probability of the observed distribution being accidental.

(S) signifies a probability of 7—5 %

S " " " " 5—1 %

SS " " " " 1 %—1 ‰

SSS " " " " < 1 ‰

No S sign indicates a probability of > 7 %.

In this table we meet only with 2 significant accumulations: bone lesion (Symptom 5) combined with dental deformity and with mental deficiency. The probability of random occurrence of these accumulations was 3.0 % and 2.2 % respectively.

On the other hand, relative antagonism is seen in 3 sets of symp-

*) Diverse symptoms — in Group I — are:

Universal adenitis (4 cases)

Atrophic appearance (5 ")

Severe anemia (1 case)

Table 8.
Survey of the Coincidence of the Symptoms in the 403 Patients of Group II (Age groups: 10—24 years)
and the Chance of obtaining these Constellations accidentally.

A	4	5	6	7	8	9	10	12	Symptom	Code	No. of patients
1 SS (5.6)	0 S (3.7)	1	1	1	3	5	2	2	Skin - mucous membrane - liver lesions	A	9
	25	2 (4.1)	4 (6.1)	1	2 S (6.9)	10 (S) (14.7)	1	5 (8.2)	Keratitis	4	42
		1 SSS (6.2)	1	1	0	7 S (3.5)	0	5 S (1.9)	Bone lesion	5	10
			6 (9.3)	0	2	2 (5.2)	2	2	Joint lesion	6	15
				3	0	3	0	3	Ear lesion	7	7
					8 (10.6)	5	1	0 **) (3.3)	Lesion of central nervous system	8	17
						11 SSS (22.4)	2	5 (7.0)	Dental deformity	9	36
							1*) S (4.4)	3	Diverse	10	7
								8 S (12.4)	Mental deficiency	12	20

*) After conclusion of the calculations it was found, cf. the first section (I, p. 277), that one patient (No. 17) was transferred from Symptom 10 (heart lesion) to symptom-free. This makes no difference, however, in the results above (the only patient with Code 10 is ruled out).

***) Cf. p. 298. This symptom of inferior mental development is excluded when the patients presented a lesion of the central nervous system.

toms: 1 + 4 S; 2 + 8 S; 2 + 9 (S). The monosymptomatic cases are fewer than expected for altogether 5 symptoms, namely: (Symptom A SS, 5 SSS, 9 SSS, 10 S and 12 S).

An analysis of the symptom formula for the 18 patients with 3 or 4 symptoms shows an exceedingly motley picture, namely: no less than 18 different formulae. It is to be mentioned, however, that in their symptom formulae the 3 patients with 4 symptoms presented the same symptoms as did some of the patients with 3 symptoms. Thus the combinations of symptoms 4 + 5 + 12 and 7 + 9 + 12 were observed twice, and 5 + 9 + 12 three times.

Extending the search for patients with the same triad to the entire

Table 9.
Symptom Formulae for Patients with 3 or 4 Symptoms.

Symptom formula		No. of patients	Occurrence of the same triad in several patients		
Group I	1+2+3	3	Group I		
	1+2+5	2	Symptom formula	No. of cases	
	1+2+10	3			
	1+3+10	1	A+5+10	4	
	1+5+10	1			
	1+3+5+10	2			
	2+3+5+10	1			
<hr/>					
Group II	1—4 yrs.	1+8+10	Group II		
	5—9 »	4+6+9	Symptom formula	No. of cases	
		4+9+10			
		9+10+12	A+8+9	2	
	10—14 »	1+8+9	4+6+9	2	
		2+8+9	4+9+10	2	
		2+9+10	4+5+12	2	
		2+10+12	5+9+12	3	
		4+5+12	7+9+12	2	
		4+6+9	9+10+12	2	
		4+7+9			
		4+9+10+12			
		5+9+12			
		7+9+12			
	15—19 »	3+6+8			
		4+5+9+12			
		4+6+12			
		5+6+12			
		5+7+9+12			
	20—24 »	6+8+10			
		1+5+9			
	36 »	2+7+12			
		2+7+8			
		5+7+8			
	43 »	7+9+10			
	44 »				

Group II, we find altogether 7 triads, each of which was observed twice (and formula $5 + 9 + 12$ was observed even 3 times) (see Table 9).

The triad is described by Hutchinson — keratitis, ear lesion and dental deformity (formula $4 + 7 + 9$) is not frequent (being observed only once, in a patient of 12 years).

The modified triad — keratitis, joint affection and dental deformity — was seen only twice; so that neither this triad can be said to characterize the clinical picture of these patients.

As emphasized by Rasch & Haxthausen*) (1939) dyads are considerably more frequent. They may be read from Table 8, when by means of Table 9 we subtract the pairs of symptoms originating from patients with 3 or 4 symptoms. Text-books emphasize the combination of keratitis with bone lesion, joint lesion or dental deformity; but they also mention that keratitis often appears as the only symptom. Among the 42 cases of keratitis in Group II, 25 presented no other symptoms, whereas the symptoms mentioned were observed 2, 4 and 10 times respectively; but this includes also the pairs of symptoms observed in 8 patients with 3 or 4 symptoms (see Table 9). Subtraction of these pairs of symptoms leaves only the presence of the dyads $4 + 6$ in 1 case and $4 + 9$ in 4 cases.

The numbers 2, 4 and 10 in group II amongst 103 patients are not high, and it must be emphasized that nowhere is accumulation seen, the said figures being slightly below the figures expected if the symptoms bone lesion, joint affection and dental deformity are found in 10, 15 and 36 patients and free combination is presumed; none of the deviations are significant.

With a larger material the correlation of the symptoms ought to be taken up again for each age-class, as the age distribution for several of the symptoms differs considerably from that of the others.

In connection with this review of the clinical symptoms it will be appropriate also to mention the deaths. Among the 304 patients with congenital syphilis, altogether 27 were found to have died*) — 13 boys and 14 girls. These deaths were found exclusively among the infants, the age distribution being as follows: under 1 month, 3 patients; 1—6 months, 17; and 7—12 months, 7 patients.

Among the dead, 10 presented no clinical symptoms of congenital syphilis; all the mothers of these 10 children gave a positive WR, and 8 of the children have themselves a positive WR, while in the re-

*) C. Rasch & H. Haxthausen: »Syphilis« in Knud Faber *et al.*'s Nordisk Lærebog i intern Medicin. 4^{ed}. Vol. I, p. 296. Copenhagen 1939.

*) After the conclusion of this work it was learned that another patient, aged 35, had died (her case history is cited in our first paper, I, p. 277).

maining 2 (negative WR in the umbilical cord blood, no examination of the serum) autopsy revealed cirrhosis of the liver, etc. (Nos. 330 and 389). Autopsy was performed on 8 out of the 10 symptom-free patients, and three unquestionable cases of syphilis were disclosed in this way, by demonstration of syphilitic osteochondritis in one (No. 302) and cirrhosis of the liver in two (Nos. 330 and 389).

Among the 17 patients who presented clinical symptoms, Symptom A (comprising syphilitic processes in the skin, mucous membranes and internal organs) was by far the most frequent symptom, being observed in 16 cases (in 12 as the only symptom, in 4 combined respectively with Symptom 5 (bone lesion) and Symptom 10 (atrophic appearance)); the remaining patient presented Symptom 5. Besides the clinical symptoms, all 17 patients gave a very strong serum reaction, and in 15 of the cases the family history gave positive information about maternal syphilis, while no information about this question was obtained in the remaining two cases. Autopsy was performed on 11 of these 17 patients and revealed: cirrhosis of the liver in 3 patients, in 2 of whom this condition was combined with hyperplasia of the spleen; in 4 other patients hyperplasia of the spleen was the only macroscopic abnormality.

As to the *causes of death in the 27 cases*, the following were recorded: Bronchopneumonia (10 cases), uncomplicated congenital syphilis (9 cases), and 1 instance of each of the following morbid conditions: convulsions, hyperpyrexia, gastro-enteritis, peritonitis, enterogenous sepsis, pulmonary hemorrhage, hemorrhagic diathesis, and whooping-cough.

26 of the children who died were born in the period of 1935—39 and are directly comparable with the 54 living patients who were born in the same years.

Clinical symptoms were equally frequent in the two groups: of the 54 living 37 (69 %) presented symptoms; of the 26 dead 16 (62 %) showed symptoms.

A tabulation of the cases after the age at which syphilis was diagnosed in the children of the two groups shows no difference between the living and the dead.

Of the 26 deaths 13 occurred too soon after the diagnosis for the institution of any treatment (3 of these 13 died from a few hours to a few days after birth).

The presence of syphilis in the mothers was fully established in 75 of the 80 cases. In the tabulation below the material is divided after the time for the diagnosis of the maternal syphilis: treated syphilis of longer standing, syphilis diagnosed in the first 7 months of pregnancy, syphilis diagnosed in the last 2 months of pregnancy, and syphilis diagnosed at the time of delivery or later yet.

<i>Maternal syphilis diagnosed:</i>	<i>Fate of the children:</i>		
	Living	Dead	Total
1 year before delivery	7	4	11
in first 7 months of pregnancy	4		4
» last 2 » » »	7	1	8
at delivery or later	32	20	52
	50	25	75

Of the 11 mothers with treated syphilis, 3 were not examined by means of the Wassermann test during pregnancy and, hence, probably not treated during pregnancy; 3 others were not treated during pregnancy.*) Of the remaining 5 mothers who received treatment during pregnancy, only 2 were treated energetically. The 4 deaths of the children in this group correspond to one untreated mother, two poorly treated and one well-treated.

The material is too small for statistical appraisal of the results of the maternal treatment with regard to life or death of the children.

Besides, as mentioned before, we lack a corresponding material of syphilitic mothers who — owing to the antisymphilitic treatment — have had perfectly healthy children.

The 27 deaths occurred in the years of 1935—1940 inclusive, and they correspond fairly accurately to the number (36) reported by the National Health Department, although in 1935 we have only 5 deaths as against 11 reported deaths — some of which correspond to children born in 1934 and, therefore unknown to us. Of course, no absolute agreement can be obtained, as in some cases it may be disputable whether the death of the child is to be ascribed to bronchopneumonia or to congenital syphilis; besides, a few deaths very soon after birth may escape registration in the syphilitic card index.

Summary.

A review is given of the correlation of the symptoms of congenital syphilis.

Over one half of the patients with symptoms are found to have presented only one symptom.

An unquestionable accumulation can be demonstrated only between bone lesion + dental deformity and bone lesion + mental deficiency. On the other hand, a relative antagonism of symptoms is demonstrated statistically several times.

Five symptoms occur alone but exceedingly seldom: lesion of the

*) The three untreated and 4 of the treated mothers mentioned above are included among the 10 mothers with a past history of syphilis to be mentioned in a subsequent section (V, p. 326).

skin, mucous membranes and liver; bone lesion; dental deformity; diverse symptoms; and mental deficiency.

27 deaths occurred in this material — all among the 73 patients who were under 1 year.

A review of the symptoms and treatment in these cases shows nothing of particular interest.

STUDIES ON CONGENITAL SYPHILIS DIAGNOSED IN THE PERIOD OF 1935—1939

IV. SIGNIFICANCE OF THE SERUM REACTIONS IN CONGENITAL SYPHILIS (DIAGNOSIS AND THERAPEUTIC CONTROL)

By *Peter Krag* and *Esther Dalsgaard-Nielsen*.

In the three preceding sections we have presented a material comprising 304 patients with congenital syphilis and have given a review of the symptoms and their correlation. Here an account will be given of the serum reactions obtained and their course in the patients who received antisyphilitic treatment.

Table 10 gives a collective survey of the serum reaction in the diagnosis of syphilis in the 304 patients. The material is divided after the strength of the serum reactions, and it is plainly evident that about 90 % of the patients have had as total result + on ordinary examination,*) and most of them have even shown a positive Wassermann and Kahn reaction higher than or equal to degree of strength 6. Table 10 further shows that there is no difference in the serum reactions of the symptomatic patients and the symptom-free.

The 32 cases in which the total result on ordinary examination was not + are investigated further:

1) The *age distribution* is striking. Of the 32 patients 15 were 52—17 years old (and all presented symptoms except the youngest one); all the remaining 17 patients were not over 4 years old, and 15 of them were not over 6 months old; more than half of them presented symptoms.

(Of the 8 negative, doubtful or non-performed reactions 7 belonged to the age-class of up to 6 months.)

This distribution of the 32 patients with a weak or negative serum reaction deviates from that of the total material, and the difference is statistically significant (the probability of it being accidental is $P \leq 0.02 \%$).

*) Krag, P.: *Acta dermat.-venerol.* 49: 528, 1938.

Table 10.
Outcome of Serological Tests.

Total result on		Symptoma- tic patients		Symptom- free patients	
ordinary examination	amplified examination				
+	+ or not performed WR and KR both ≥ 6	180	81.8	68	80.9
+	+ or not performed WR and KR both ≥ 3 but one ≤ 5	16	7.3	8	9.5
\pm }	+	6 }	6.8	1 }	4.8
\pm }	not performed	9 }		3 }	
\pm }	\pm	2 }	3.2	1 }	3.6
- }	\pm	2 }		0 }	
Negative, ? or not performed		5		3	

From these findings the conclusion will be: that the age-classes of 5—19 years nearly always give strong serum reactions.

As the syphilitic card index and, thus, our material have only poor possibilities of registering any possible cases of parenchymatous keratitis giving a negative serum reaction,*) we have gone through 114 cases of acute parenchymatous keratitis which were diagnosed in the Eye Clinic of the Rigshospital in the period of 1926—42.***) Of these patients 98 gave + WR, 11 were seronegative, and 5 were not examined serologically.

For a number of years, in the State Serum Institute, human heart extract was employed for antigen in the Wassermann test, but in 1933 this antigen was replaced by cholesterinized calf heart extract (Mørch). The significance of this change is plainly evident from the following:

In 1926—32: WR on 68 keratitics gave 11 seronegative;
 » 1933—42: » » 41 » » 0 »
 (The difference is significant, $P \leq 0.6\%$.)

As our material comprises the seropositive cases from the entire country for the period of 1935—39, we reckon that hardly any case of keratitis has been missed.

2) *The connection between the symptoms and the weaker serum reactions* is quite in keeping with the age distribution, as Symptoms A, 4 and 7 were found particularly often in these cases.***)

It is to be mentioned that in these patients some of the symptoms

*) Cf. Article I, p. 271.

**) We are greatly obliged to Professor Henning Rønne, M.D., for permission to make use of the case records in the Eye Clinic of the Rigshospital.

***) Accumulation only of Symptom 7 (ear lesion) is statistically established; $P = \text{about } 2\%$.

have to be considered scar-like; in particular three-fourths of Symptom 4 in this group are merely sequelae of keratitis.

In reviewing the individual symptoms it was emphasized that the diagnosis of syphilis was always made on a suitable combination of serum reaction, symptoms and family history.

In the cases of suspected patients or patients with a weak serum reaction, however, it is to be emphasized that each of the 24 symptomatic patients presented 1—5 symptoms and that 16 in addition gave a positive family history of syphilis. The 8 patients who were merely symptomatic presented the following symptom formulae: No. 5, Symptoms 4 + 6 + 7 + 8; No. 15, 4 + 7 + 9 + 10; No. 19, A + 4 + 5; No. 23, 4 + 7 + 8; No. 41, 4 + 9; No. 76, 7; No. 82, 4; No. 388, 1 + 2. So even 5 of these patients presented 1 or 2 scar-like symptoms, they all had manifest symptoms.

All the 8 symptom-free patients gave a positive family history of maternal syphilis; 4 of them gave also information about syphilitic sibs; 2 died (autopsy diagnoses: 1) cirrhosis of the liver and 2) atrophy, anemia, congenital syphilis); 1 gave repeatedly \pm WR during the first 6 months of life; and the last (No. 377) was given anti-syphilitic treatment immediately after the first WR — with excellent effect, according to information from the physician.

For all 304 patients we have tried to obtain information about the antisymphilitic treatment given, with details about dosage and duration of treatment.

It was found that 263 patients (86.6 %) were given at least one series of treatment (mercury, neosalvarsan, bismuth, or other remedies; 3 (1 %) were given malaria therapy, sulfosin or colon vaccine + mercury; and in 6 (2 %) a treatment was instituted but soon discontinued.

8 patients (2.6 %) were not given any treatment. In 24 cases the respective physicians failed to answer the question concerning treatment: In 13 of these 32 cases, however, the patient died so soon after the diagnosis of congenital syphilis that any treatment could hardly have been instituted (the 3 highest lifetimes reckoned from the day of diagnosis are 8, 13 and 17 days).

Among the patients who were over 1 year old the various symptoms were distributed rather equally, so that in 1935—39 there has been no tendency to omit antisymphilitic treatment in cases of keratitis.

Result of 1—5 Years' Serological Observation of the Patient Material under Antisyphilitic Treatment.

By means of the serum reactions it is practicable to some extent to estimate the therapeutic results. As expressions for the effect of

the treatment we have here employed the rest reaction and the fall in the strength of the reaction.

As the material consists of new-diagnosed cases, the observation periods are short so that only the temporary effect may be estimated; whether permanent results (guarantee against clinical-serological relapse and late manifestations) cannot yet be decided.

Cases with indefinite information about the treatment are ruled out. The cases are reckoned as being observed only in those years in which results of serum reactions are available; and the course of the serum reactions is analyzed thoroughly only in those cases where the initial reaction was at least $WR = KR = 6$, as 20 cases with weaker reactions will be mentioned later on (p. 319).

So, for the individual years of observation only the following numbers of cases are serviceable:

1 year, 141 cases; 2 years, 99 cases; 3 years, 53 cases; 4 years, 35 cases; and 5 years, 9 cases. Of course, a well-observed patient may be recorded several times — as, for instance, under 1, 2, 3 and 5 years' observation.

As the 141 cases under observation after 1 year of antisymphilitic treatment are fairly surveyable as to the number of series of treatment, they will be dealt with first, while the smaller but longer observed materials will be mentioned later.

By means of point diagrams the following variables are correlated by pairs: the initial reaction; the age of the patient, the number of series given, and the rest reaction; in several cases it has been practicable by means of a code to insert a third variable per diagram. The diagrams are not reproduced here, but Table 11 gives all the information derived from the diagrams, as the various groupings took place on the basis of the diagrams.

Initial Reaction. — This is the average of WR and KR for the last serum reaction prior to the institution of the treatment. It was found that in the range of 7—13.5 all degrees of strength of reaction were amply represented by 118 of the 141 cases, whereas only a few patients gave a weaker initial reaction, while some gave a stronger.

Age. — The age of the various patients is recorded in one of the following 5 groups: ≤ 1 , 1—9, 10—14, 15—19 and ≥ 20 years — unfortunately the infants are represented but scantily.

Treatment. — Here the treatment is recorded as ≤ 1 , 2 and 3 »cures«, 2 of the patients in the last group receiving 4 cures (here 1 »cure« means one period of treatment of 1—2 months, regardless whether the remedy employed was neosalvarsan, bismuth or mercury, or possible combinations of these).

Rest Reaction. — This is the average of WR and KR at the end of the observation period. It is to be mentioned that under »examination after 1 year« we have reckoned all patients who were examined

Table 11.

Rest Reactions after 4 Year of Antisypthilitic Treatment.

Connection between the rest reaction and the initial reaction, age of the patient and intensity of the treatment.

Age of patient initial reaction*)		0			1—9			10—14			15—19			20—			Total
No. of cures	Rest reaction	+	++	+++	++++	+	++	+++	++++	+	++	+++	++++	+	++	+++	++++
≤ 1	≥ 6.5										1			1			9
	5.0—6.0											1			1		7
	3.5—4.5											1			2		6
	2.0—3.0		1				1										26
	0.5—1.5										1						2
2	0																1
	≥ 6.5				1												25
	5.0—6.0				1						1	7			2	1	17
	3.5—4.5					2	3	1			1	2			2	2	4
	2.0—3.0							1			1				1	1	61
≥ 3	0.2—1.5					2					1						4
	0					1	1										2
	≥ 6.5																9
	5.0—6.0					2	1										14
	3.5—4.5				1						1	3			2		12
Total.....	2.0—3.0							2	3		1	1		1	1		8
	0.5—1.5							1			2	1					54
	0	1				2	3								2		9
	≥ 6.5																6
	5.0—6.0	1	4	4	5	14	22	3		10	19	5		1	10	8	141

*) + = 6.0—6.5 degrees of strength; ++ = 7.0—9.5; +++ = 10.2—12.5; ++++ = 13.0.

Table 12.
Rest Reaction after 1 Year of Antisymphilitic Treatment.*)

Rest reaction	No. of cures			Age of patient			Initial reaction				Total
	≤ 1	2	3	0	1-9	≥ 10	+	++	+++	++++	
6.5—	9 (34.6)	25 (41.0)	14 (25.9)	1 (7.1)	10 (25.6)	37 (42.1)	0	10 (20.4)	29 (42.0)	9 (45.0)	48 (34.0)
3.5—6.0	13 (50.0)	21 (34.4)	20 (37.0)	1 (7.1)	14 (35.9)	39 (44.3)	1 (33.3)	22 (44.9)	26 (37.7)	5 (25.0)	54 (38.3)
0 —3.0	4 (15.4)	15 (24.6)	20 (37.0)	12 (85.7)	15 (38.5)	12 (13.6)	2 (66.7)	17 (34.7)	14 (20.3)	6 (30.0)	39 (27.7)
Total	26	61	54	14	39	88	3	49	69	20	141

*) Extract of Table 2 for the three combinations of variables showing a noteworthy coincidence (No. of cures, age of the patients, and degree of the initial reaction in relation to the rest reaction). For each of the three variables (divided into 3-4 groups) the frequency of the three degrees of the rest reaction is recorded — both the number of cases and the percental frequency. Employment of italics or bold type indicates that the frequency is respectively lower or higher than the corresponding frequency found for the total material.

10—14 months after the institution of treatment; but we have not tried to calculate the reaction for patients, for instance, who were examined 8 and 16 months after the commencement of treatment.

Many of the squares in Table 11 are occupied but very poorly, so that it is obvious that the longer observed but smaller material does not lend itself to this sort of grouping.

From Table 11 (or from the diagram) the figures are gathered for each pair of variables, as for the present we leave the other variables out of consideration. Thus we find that the number of cures, the initial reaction and the age show no tendency to mutual relation, whereas there is a distinct connection between the rest reaction and all the 3 other variables, as a low rest reaction is particularly frequent in infants and young children, in patients who have received 3 cures or more, and in patients with a low initial reaction.

These three relations of the variables, which all may be deduced readily from the findings recorded in Table 11, are presented collectively in Table 12, from which it is plainly evident that the age of the patient is most strongly connected with the magnitude of the rest reaction.

As the undertreated cases (≤ 1 cure) and cases with particularly weak or strong reactions perhaps have a disturbing effect on these accounts, the analysis has been repeated for the 95 well-treated cases showing an initial reaction of 7.0—12.5; and even though this material is smaller than the preceding, it illustrates very well the relations mentioned.

In Fig. 3 the analysis of the 95 cases is presented graphically in 6 columnar diagrams which illustrate very strikingly the great frequency of a low rest reaction in the infants and younger children. Besides, the diagrams show that the low rest reaction also — though in a lesser degree — is dependent upon the number of cures the patient has received, and, finally, that a high rest reaction is particularly frequent in cases with a high initial reaction. It may be added that these columnar diagrams greatly resemble the unpublished columnar diagrams for the total material.

Finally we have divided the 95 cases into 4 groups — each with its separate combination of initial reaction and number of cures (++ and 2 cures; ++ and 3 cures; +++ and 2 cures; +++ and 3 cures). In each of these small materials we also find the youngest patients show the highest frequency of low rest reactions. In three of the cases this accumulation is even significant, as the probability of its appearance being accidental is $P = 1.3$, $P = 4.2$ and $P = 1.4$.

It is to be emphasized that the difference of the rest reaction in patients treated with 2 cures and in patients given 3 cures is rather

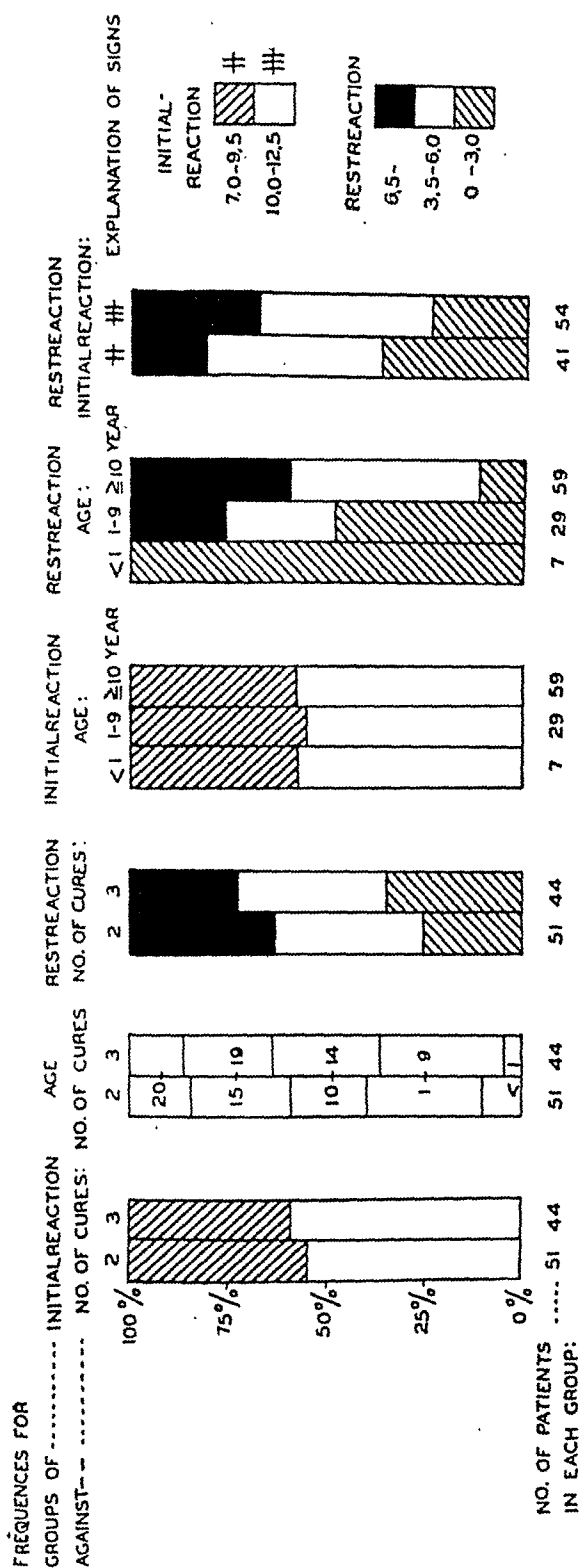


Fig. 3.

Six columnar diagrams, one for each combination of the 4 variables (age, initial reaction, number of cures, and rest reaction). The diagrams show the percental frequencies for groups of one variable as against 2-3 groups for the other variable.

slight, as a rest reaction ≤ 3 was seen after 2 and 3 cures respectively in 25 % and 37 % of the patients. — Presumably the initial reaction plays a little greater role: a rest reaction ≤ 3 was seen after initial reactions of 7—9.5 and 10—12.5 in respectively 39 % and 20 % of the cases.

The age is the most important variable; the frequency of rest reaction ≤ 3 for the five age-classes here employed was as follows: for infancy 86 %; for 1—9 years 38 %; for 10—14 years 12 %; for 15—19 years 18 %; and for ≥ 20 years 10 %.

The material is too small to allow of an X^2 calculation for the distribution tables corresponding to Table 11, but after summations of the table to four-spaced tables, significance was demonstrated frequently (cf. above).

With a subsequent (larger) material the question will be taken up again.

Of the 20 excluded cases with weak initial reaction 18 were re-examined 1 year after the commencement of treatment. Of these patients 9 were under 1 year — and they all became seronegative, although only 3 had received 2 cures or more. Of the remaining 9 patients 7 were over 15 years; and although 5 of these 9 patients were given 2 or more cures, only 3 became seronegative. (As a matter of fact, the initial reaction was a little lower in the older patients.) The probability of the above-described distribution being accidental is slight, $P = 0.5$ %.

Accordingly, the experiences from the 1-year material may be summarized to this effect: that a low age is of the greatest importance to a good therapeutic result.

The fall in the strength of the reaction is investigated in a similar manner as the rest reaction — by correlation with the three other variables. A marked fall was found to be most frequent in patients given 3 cures or more, in patients with a high initial reaction and in very young patients. As the initial reaction here plays a greater role than in the investigation of the rest reaction, elucidation of the question is more complicated, and hence the fall in the strength of the reaction is considered unsuitable for a description of the serological course of the cases.

Of the 141 patients who were under observation for 1 year 44 were symptom-free. A review of these cases by means of point diagrams shows that this group did not in any way differ from the total material.

A subdivision of the symptomatic patients into diagnostic groups shows that it would hardly be practicable to appraise the peculiarities of the course of the cases because of the size of the material, the age

distribution in the various groups, accidental differences in the intensity of the treatment — and, finally, the circumstance that many patients presented several symptoms.

From year to year in the observation period, as a rule, there is an increase in the frequency of low rest reactions and of seronegative cases. In the observation periods of 4 and 5 years the cases are so scanty that they may hardly be appraised. Furthermore, it is to be expected that refractory cases with a persistent positive serum reaction (with this method of accounting) relatively will stamp the picture too strongly after 3 years of observation, as these patients are more likely to return for control and treatment, whereas the patients who became seronegative after 2—3 years of treatment more likely may fail to return for subsequent control.

In the materials which have been under observation for at least 2 years the treatment was carried through in a highly variable manner. For illustration, it may be mentioned that of the 99 patients in the 2-year material 18 received up to 1 cure in the first year, while the remaining 81 (well-treated) patients received a total of 2—6 cures, as 8 different schemas of treatment were employed (minimum: 2 cures in 1' year, none in 2' year; maximum: 3 cures in 1' year, 3 in 2' year). Therefore, the individual groups which were given about the same treatment are too small for a detailed analysis.

Concerning patients observed for 3, 4 and 5 years it may be mentioned that 60—80 % were well-treated.

As to the materials under observation for at least 2 years we have to reckon that they comprise in particular the patients who respond to the treatment but slowly; for there is a distinct tendency to discontinue the treatment and serological control when seronegativity is obtained and verified through 1—2 years.

For the well-treated patients with an initial reaction of 7.0—12.5, Table 13 gives some data for the 2' and 3' years of observation, illustrating the behavior of the rest reaction in the various age-classes, leaving out of consideration the number of cures given and the initial reaction.

From the figures recorded in Table 13 it will be noticed that after 2 and 3 years of observation of the well-treated patients with an initial reaction of 7.0—12.5, the number of infants (and young children) is too small to furnish any proof of a connection between the age of the patient and the magnitude of the rest reaction.

Comparison of the 1-, 2- and 3-year materials shows some increase in the frequency of a low rest reaction. It is to be regretted, however, that in spite of 3 years' treatment there still are 13 out of 36 patients who show a rest reaction ≥ 3.5 ; and this poor outcome has to be considered on the background of the fact that among 14 infants with an observation period of 1 year only 2 showed a rest reaction ≥ 3.5 .

Table 13.

Frequency of Groups of Rest Reaction in Relation to the Age Groups.

Rest reaction	2 years' observation				3 years' observation			
	0	Age 1—9	≥ 10		0	Age 1—9	≥ 10	
6.5—	1	1 (6.3)	9 (21.4)	11 (17.2)			3 (12.0)	3 (8.3)
3.5—6.0		7 (43.7)	11 (26.2)	18 (28.1)		3 (33.3)	7 (28.0)	10 (27.8)
0 —3.0	5	8 (50.0)	22 (52.4)	35 (54.7)	2	6 (66.7)	15 (60.0)	23 (63.9)
	6	16	42	64	2	9	25	36

Conclusion: The material shows a demonstrable connection between the tendency to a fall in the strength of the reaction and the initial reaction, the intensity of the treatment and, especially, the age of the patient. The material is too small for an inquiry into the relation between the falling tendency of the reaction and the symptoms presented by the patient.

The material includes 16 patients in whom seronegativity was obtained strikingly soon, within 1 year.

In all these patients the serum reactions were strong. The 5 weakest reactions lay between $WR/KR = 7/6$ and $9/10$, while the remaining reactions were distributed equally over +++ and ++++.

The age distribution of the 16 patients showed a distinct preponderance of infants: 3 patients were 15—16 years, 1 was 9 years and 3 were 6—12 months, 6 were 1—5 months, and 3 were under 1 month.

In 6 of the 12 infants the serological diagnosis of syphilis was corroborated by typical skin symptoms, as a rule also by processes in the mucous membrane, and 2 other patients presented respectively periostitis of the arm and enlargement of the liver + spleen. In the 4 symptom-free infants the diagnosis was supported by the demonstration of syphilis in the mother. In these 4 patients the course of the serum reactions showed repeatedly positive results and/or a gradual fall in strength, so that the possibility of a serum reaction transmitted from the mother was to be considered only in the case of one child, 1 year old. In this patient the reaction fell in 3 months from $W/K^* = 11/10$ to $1/1$ while the child was receiving energetic treatment (inunction with mercury ointment and injection of calomel, 1 cg. $\times 3$), but the duration of the treatment is not recorded. The rate of the fall in strength of the reaction in this case was not more rapid, however, than that observed in 2 children, 3 and 5 months old, with syphilis of the liver, etc., and the spontaneous disappearance of the trans-

*) see p. 327 this paper.

mitted serum reactions takes place within the first months of life. So, in this case, we have to consider the diagnosis fully established.

In the cases of the 4 school children, who were from 9 to 16 years old, the diagnoses were: 1) slight joint lesions localized to the knees that subsided under treatment; 2) universal adenitis, hypertrophy of the tonsils, enuresis nocturna; 3) moderate mental deficiency; 4) dyspepsia — in an atrophic-looking boy of 16 years with a sedimentation rate of 64 mm. In these 4 patients the fall in the strength of the serum reaction proceeded more slowly; yet seronegativity was obtained in about one year after the time of the diagnosis — after repeated positive tests which showed that each of these four patients were suffering from syphilis.

All 16 patients were given antisyphilitic treatment; 11 of them were given both neosalvarsan and bismuth (at least 1 series of injections with each remedy, and 8 of them received even 2 series of each remedy). Still, series of actually combined neosalvarsan and bismuth cure were given only to 7 patients (5 of whom received two series, while 2 received three series). 2 patients were given only neosalvarsan (infants with symptoms on the skin and mucous membranes). The remaining 3 patients were given other therapeutic combinations: spirocid + several bismuth series; neosalvarsan + calomel; mercurial inunctions.

In the 7 patients who were given the most energetic treatment the seronegativity was not obtained sooner than in the remaining 9 patients.

Conclusion: According to the data above, there is no reason to think that among the 16 patients who showed a decisive fall in the serum reaction within 1 year after institution of treatment there were some cases which reasonably might be suspected of having given an unspecific reaction or of representing a spontaneous disappearance of antibodies transmitted from the mother.

The present material includes some patients who responded to the treatment but poorly. The following cases are mentioned.

- a. 8 patients who 3 years after the commencement of treatment showed a serum reaction ≥ 6.5 .
- b. 11 patients who 4 or 5 years after the commencement of treatment showed a rest reaction ≥ 3.5 . It is to be mentioned, however, that these two groups comprised only 17 patients altogether.

The explanation of these poor results is in some cases to be found in the scanty and hesitating antisyphilitic treatment. In other cases the patients were over 20 years old, or the initial reaction was particularly high. But in 6 of the 17 patients the lesion was diagnosed at an age of 8—20 years; these patients showed an initial reaction of 9.5—13.5, and they were all given a thorough treatment: 2—3 cures in the first year, and altogether 5—6 cures in the first three years; at least three of the cures were combined (neosalvarsan + bismuth).

From this it is evident that the chance of a favorable therapeutic result is not so good when the patient has reached school age or an older age-class. It may be added that in these cases the physicians (sometimes the patients) have given up obtaining seronegativity and have discontinued the treatment and observation after 6—8 cures.

Summary.

The frequency of the various levels of serum reaction is described. Particular mention is made of a few cases with a strikingly weak reaction.

Data are given on the treatment of the 304 patients, with particular mention of those who were well treated and well observed serologically. The fall in the strength of the reaction is investigated in relation to the age of the patient and the initial reaction for the observation periods of 1, 2 and 3 years. The intensity of the treatment given to these groups is recorded. Early treatment increases the chance of obtaining seronegativity rapidly.

This investigation cannot be carried through for each group of symptoms.

Particular mention is made of cases with a rapid fall in the strength of the reaction and cases with a slow fall.

STUDIES ON CONGENITAL SYPHILIS DIAGNOSED IN THE PERIOD OF 1935—1939

V. CONGENITAL SYPHILIS IN RELATIVES (SIBS AND MOTHERS — CHILDREN)

By *Peter Krag and Esther Dalsgaard-Nielsen.*

In the preceding four sections a review is given of the symptoms, serum reactions and treatment of 304 cases of congenital syphilis. The material includes several patients who are sibs — and an attempt is made, therefore, to elucidate the appearance and extension of congenital syphilis in the families on which sufficient data are available.

In the following three tables the family groups are described in relation to the child (in the material) on whom the diagnosis was first made, the grouping taking place after the number of sibs found in the material. Tables 14, 15 and 16 give information respectively about the maternal syphilis, sibs in the material and sibs outside the material.

Table 14 shows that the information obtained about 30 % of the mothers is very defective, as the answers in the questionnaire concerning this point are lacking (22 %) or say: (presumably) well, WR not performed (4 %); old syphilis, without particular data (4 %).

Concerning the 179 cases in which adequate information was obtained, it is a striking fact that *three-fourths of the mothers (71 %) did not have their syphilis diagnosed till the child was born*; in more than one-half of these cases (39 %) the diagnosis was not even made till the presence of syphilis had been diagnosed on the child. This appears to imply the basis for an extension of the serological control early in pregnancy. Control examination in cases of pregnancy has given the diagnosis in about 7 % of the mothers in this material, but here we lack the corresponding figures which should tell us how often the earliest diagnosis and treatment of syphilis in a pregnant woman may prevent the appearance of congenital syphilis in the child. But as the cards accompanying the blood samples for Wassermann tests

Table 14.
Data on 256 Mothers and their Syphilis Tabulated after the Number
of Children in the Material.

		No. of children in the material per family group					Percent- distribution of all mothers	Percent- distribu- tion of cases with detailed data (179)
		1	2	3	8	Total		
Syphilis diagnosed prior to birth of the child	No information....	55	1	1		57	22.3	30.1
	Mother well or pre- sumably well	10				10	3.9	
	Mother syphilitic, details lacking...	8	2			10	3.9	
	> 3 years	16	2	1	1	20	7.8	11.2
	3 years — 9 mths...	8	1			9	3.5	
	9—0 mths.....	12				12	4.7	6.7
Syphilis diagnosed at birth		11				11	4.3	6.1
Syphilis diagnosed after delivery	before diagnosis on the child.....	46	9	3		58	22.6	49.6
	after diagnosis on the child.....	55	12	2		69	27.0	
	Total mothers.....	221	27	7	1	256		38.6
Total children in the material.....		221	54	21	8	304		

do not require any information about pregnancy, a card index material will not be able to give any information about the good therapeutic results obtained in pregnant syphilitics — and only the cases with unfavorable results present themselves here.

Finally, the material includes 29 mothers who ought not to have got children with congenital syphilis as the maternal syphilis was diagnosed more than nine months before the birth of the child.

The interval between the diagnosis of maternal syphilis and the birth of the child varied greatly, being less than 2 years in 8 cases, 3—7 years in 16, and over 9 years in 5. Of the children 9 were under 1 year at the time of the diagnosis of their syphilis, so that the treatment in their cases could be instituted at a favorable juncture. But 16 children were over 10 years old at the time of the diagnosis and, consequently, in age-classes which respond less favorably to the treatment.

The 29 children of previously treated mothers are distributed like the total material in the various symptoms groups. There were 9 infants with 4 deaths, while the total material has 27 deaths among 73 infants.

Concerning 16 of these 29 mothers the card index has given information about control tests on the mothers prior to the birth of the child. Thus it is found that on 3 mothers no test had been made during the 6—14 years gone since the diagnosis of syphilis first was made, and that on 8 mothers no test had been made from 1½ to several years before the birth of the child, while 5 were examined during pregnancy, as a rule 6—7 months before delivery. Of these 5 mothers, 4 were seropositive, the fifth seronegative (with the less sensitive reactions employed in 1922).

Regarding 10 of these 16 mothers, information about their treatment was obtained from their respective physicians at that time: 6 were not given any antisyphilitic treatment whatever; 2 received treatment only for the first month, 1 only in the last month of pregnancy, whereas 1 was given a good deal of myosalvarsan before and during pregnancy. The children of the first two treated mothers had distinct clinical syphilis; the children of the last two mothers showed merely + WR; but the first two children and the last one died before they were two months old.

As a rule, the patient had evaded control (and treatment) at such a late juncture of the cure that the physician undoubtedly contented himself with sending her an ordinary summons to return for treatment, without trying to trace her through the police as the risk of direct infection now was very slight.

If the respective physicians have instructed the patients that they ought to return for control examination and cure in case of subsequent pregnancy, such directions were not complied with.

As the children showed no seroresistance during the antisyphilitic treatment in the first years of life, there may hardly be any reason to assume that the spirochaetes of these mothers have been particularly chemoresistant.

The serum reaction of several of these mothers is known. Of the 29 mothers whose syphilis was diagnosed prior to the birth of the child the majority were seropositive (total result + in 19, ± in 6, — in 4; the amplified test, which was performed only in 2 of the 4 seronegative cases, showed the total result ±). Strong serum reactions were found especially in the mothers with a long interval between the syphilis diagnosis (and treatment) and the birth of the child.

In some of the other groups too the WR results are of interest:

1. The present material includes 10 patients in whose family history it merely says that the mother was well or presumably well. All these patients presented unquestionable clinical signs of congenital

syphilis, and the serum reactions were strong except in the case of one man (No. 15), aged 43, who presented signs of a previous parenchymatous keratitis, besides choroiditis diss., deafness and Hutchinson teeth. In 1935 his serum reaction was $W/K^*) = \frac{1}{4} -$, and in 1936 it was $-/- ++ + \pm$. He had never received any anti-syphilitic treatment, as the diagnosis was not made till he was 43 years old; there was no information about WR in the mother, but she was said to be well.

In the next 5 of the 10 patients, the mother was claimed to have —WR, but this could be verified through the syphilitic card index only in 3 of the cases. In the last 4 cases it was merely stated that the mother was well; but in 2 of these cases the father is recorded as having syphilis, and in one of these cases two sibs are stated to have died of cerebrospinal syphilis in a hospital for mental diseases.

5 of the patients are from 14—43 years old, which perhaps explains why the treating physician had no information about maternal syphilis. On the other hand, obviously it is due to an accidental defect in the records when the mothers of children, $\frac{1}{2}$ —13 years old are unknown to the physician with regard to the presence of syphilis.

That WR in the mothers sometimes is recorded as negative is not particularly surprising, as the serum reaction very well may disappear spontaneously in the 10—15 years that have passed between the parturition and the examination.

2. Table 14 further shows that in 11 cases the maternal syphilis was diagnosed at the birth of the child — and to this it is to be added that in 7 additional mothers the syphilis was first diagnosed in the puerperium.

All these 18 mothers gave strong serum reactions (the 2 weakest were $W/K = 5/3$ and $7/5$). Often the Wassermann test on the children was performed somewhat later on, so that no direct comparison as a rule is practicable. In 5 cases comparable results were obtained in the first week after birth, and here the reactions of the children were found on an average to lie 1—2 degrees lower than those of the mothers.

In 10 of the children Symptom A was the only symptom; one child presented Symptoms A and 10 (small and delicate), and one was later found to be mentally defective. The serum reactions for these 12 symptomatic children differed somewhat: 7 showed unquestionable serum reactions, at least $W/K = 5/2$, as WR often is stronger than KR; in 2 cases the amount of serum was too small for the titration; and in the remaining 3 cases the serum reactions

*) $W/K=1/-$ indicates: $WR=1$ and $KR=0$; this abbreviation will be employed in the following.

were weak ($W/K = u/\geq 1$, —/— and —/u)*). In 2 of these cases, however, strong reactions were obtained 2—3 months later, whereafter treatment was instituted; in the third case (No. 395) the patient had a bullous syphilid; the presence of *spirochæte pallida* was demonstrated, and treatment was instituted at once.

As our material has no data on the treatment of the mothers, nothing would be gained by comparing the course of the serum reactions in the mothers and children.

Concerning the 104 mothers in this material who were registered in 1937 or later we have information about the history of the patient, the clinical diagnosis and the possible presence of syphilitic symptoms.

Anamnestic data on the presence of syphilis were found only in 34 of these cases: syphilis diagnosed previously (12 cases); husband suffering from syphilis of long standing (13); abortion or stillborn children (6); and possibility of infection (3).

Clinical signs of syphilis were found only in 10 of the remaining 70 patients: old syphilis in 6, recent (secondary) syphilis in 4.

Of the 60 patients in whom the diagnosis was based entirely on the serum reaction and the presence of syphilis in the child, the absence of symptoms was verified in 41 cases by the answers to the questionnaire, while in 19 cases we had to be content with the information furnished by the Wassermann cards which were filled in well.

Concerning mothers who were registered prior to 1937 the clinical data are so few that no detailed analysis of this part of the material is practicable.

It is to be emphasized that 74 %, of the 133 mothers in whom syphilis was diagnosed about the same time as in the children were not over 40 years old, *i. e.*, the treatment was indicated not only for the sake of the mothers themselves but also for the sake of eventual later children (50 % of these mothers were 32 years or younger).

A description of the family aspects of the 304 patients naturally requires also some mention of their sibs in this material and outside.

Table 15 gives a survey of the 35 patients with one or more sibs in the material.

As will be noticed from Table 15, in nearly one half of the cases the lesion of the first-diagnosed patient was diagnosed too late to ensure the younger sibs against congenital syphilis. The distribution of the sibs in symptomatic and symptom-free patients shows no relation to the symptoms in the first-diagnosed children.

Particular mention is to be made of a family from the country, of whose members 8 are included in this material, while 2 (outside the material) are under observation for syphilis (see p. 333).

*) u means unreadable reactions.

Table 15.
Survey of the 35 Patients with Sibs in this Material.

No. of patient	No. of sibs	Symptoms of patient	No. of sibs		No. of sibs		Total
			older	younger	with symptoms	without symptoms	
6	1	0	2	4	3	3 ⁰⁾	6
21	"	+	11	10	12	9	21
2	2	0	4		2	2	4
5	"	+	3	7	4 ⁰⁾	6	10
1	7	0	7		3	4	7
Total 35			27	21	24	24	48

⁰⁾ 46 sibs gave a strong + WR; the two marked with ⁰⁾ showed only a total result of \pm .

In 27 cases in the material (see Table 15) syphilis was diagnosed in two sibs. As was to be expected, the presence of symptoms (and often several symptoms) was encountered somewhat more often in the sib whose syphilis was diagnosed first.

In the first-diagnosed sibs Symptoms A, 4, 5 and 6 were slightly predominant. This is quite natural, as these very symptoms are suggestive of syphilis and thus lead on to a Wassermann test and the diagnosis together with examination of the family (but the difference is not at all significant).

There was no particular parallelism between the symptoms in the sibs: symptom-free sibs were encountered 4 times; keratitis once; adenitis twice; and mental deficiency 5 times. The last figure may seem striking, but in two of the cases the presence of Symptom 12 in one sib was undoubtedly a result of the concurrent Symptom 8 (neurosyphilis) — as mentioned already in our first paper (I, p. 278). There is nothing striking in the occurrence of three sets of imbecile sibs (the probability of their accidental appearance is about 8 %). These pairs of sibs suggest that the family factor may be of some significance to the coincidence of congenital syphilis and mental deficiency.

Further analysis of the symptoms in the sib groups is not practicable, however, as in judging of the frequency of the various symptoms we should have to take into account the individual age-classes, and thus the small sib groups would be subdivided even into small units.

A complete account of the sibs outside the material cannot be worked out on a material which has been under observation only for 1—6 years (the questionnaires were answered in 1940—41), as presumably several of the younger mothers would have more children after 1941. So in several cases our data on the size of the families

will merely be temporary. Furthermore, in 98 of the 256 families it was not possible from the questionnaire*) with certainty to decide whether the patient was an only child or whether the physician in filling the questionnaire was lacking information about the sibs of the patient. More likely, however, at least one-half of the 98 patients were only children in 1941; for 44 families we know with certainty that the patient was an only child.

Table 16.

Sibs outside the Material in 114 Cases of Patients with Detailed Information in their Records.

Sibs outside the material	24 symptom-free patients	90 symptomatic patients
Abortus or stillborn	1	20
Died in infancy or early childhood	3	> 34
Died at an older age		9
Sibs not examined or described	4	> 32
Reported well or living		> 52
Wassermann negative	34	> 104
Observation for syphilis	7	4
Syphilis diagnosed	4	18
Total No. of sibs	53	> 270

Table 16 gives a survey of the information received about the 114 patients of whom we know that they had sibs outside this material. In the complete material, which temporarily is unobtainable, there will presumably be a few more syphilitic or suspect children, but considerably more children who are perfectly well. In 4 cases, for which the number of sibs could be given merely as »several«, we have reckoned with 4—12 sibs.

In three cases we have definite information about single members of these groups of sibs, so that the total of the schema (> 326) is greater than the total number (> 323).

From Table 16 it will be noticed that the symptom-free patients have not so many sibs as have the symptomatic patients. Presumably this is due to the fact that the symptom-free patients are younger, being almost completely absent from the age group of 20 years or more.

*) In the questionnaire we asked for information about all sibs; for those who were well we wanted the year of birth and the result of WR; for the syphilitics we wanted the card index dates, symptoms and treatment. Unfortunately the questionnaire did not contain the direct question: »Has the patient any sibs?«

Syphilis was diagnosed in the cases of 22 sibs; and it is reasonable to assume that it was present in a considerable part of the 21 abortuses and of the ≥ 37 sibs who died in infancy or early childhood — altogether, presumably, 60—70 sibs.

It is rather striking that among the 53 sibs of the 24 symptom-free patients only 1 abortion and 3 deaths are recorded whereas 20 abortions and over 34 deaths are recorded for the 270 sibs of the 90 symptomatic patients. But, as the symptom-free patients are younger and have fewer sibs, it would not be warrantable from these figures summarily to conclude that syphilis is particularly malignant in certain families, even though the probability of the observed distribution being accidental is $P = 3.2\%$.

As a general result of this investigation it may be mentioned that the total 156 mothers have had at least 675 children and that about one-half of these children are known with certainty to have been syphilitics (the 304 children in this material and > 22 outside the material).

Only for 140—150 children have we information about a negative WR and thus almost a certainty that they are non-syphilitic.

On reviewing the syphilitic sibs outside the material it was found that in two of them syphilis had been diagnosed in 1935 so that they really ought to have been included in this material; but the information received about these two patients in 1935 on the cards accompanying their blood samples caused them to be registered in the card index as cases of recent acquired syphilis, based entirely on the serum reaction (men, 22 years old), and this matter was cleared up so late that we did not wish to add these two unquestionable cases of congenital syphilis to the 304 in the present material. (See also our first paper — I, p. 271 — where two other patients who erroneously were omitted, are mentioned.)

Our material, as mentioned, includes several sets of sibs, namely: 27 sets of 2 sibs, 7 sets of 3 sibs, and 1 set of 8 sibs. In the following some of these sets of sibs will be mentioned in detail.

Among the group of 2 sibs in this material a few sets have to be mentioned separately, namely: Nos. 74 + 48 and Nos. 97 + 78.

The first set of sibs (Nos. 74 + 48) came from a family in which there were 4 older children in good health and a younger brother who was said to have congenital syphilis (card index dates not given). Syphilis was first diagnosed in the older of the two brothers when he was admitted to the Bispebjerg Hospital for orchitis, presumably of syphilitic character; he presented no other signs of congenital syphilis. The younger brother was suffering from dementia paralytica and was given malaria therapy in the St. Hans Hospital. No information could be obtained about the parents.

Nos. 97 + 78 had also an older brother with congenital syphilis. Besides, there were positive data on syphilis in the mother, but this

was diagnosed first at the same time as the lesion in No. 78 — 3 weeks after syphilis had been diagnosed in the youngest child (No. 97), who at that time was admitted to a surgical clinic on account of *bilateral hydrarthrosis of the knee and parenchymatous keratitis*. The brother (No. 78) presented no symptom other than slight imbecility.

After this, the groups with 3 sibs in the material are to be mentioned briefly.

Nos. 103 + 77 + 86: None of these sibs presented any definite sign of congenital syphilis; in one the teeth were a little suspect. The presence of syphilis was discovered accidentally while No. 103 was staying in a medical clinic. Syphilis in the mother had been ascertained previously; now she was suffering from *tabes dorsalis* with optic atrophy.

Nos. 150 + 39 + 44: In this case no information was available about syphilis in the parents. The disease was diagnosed first in No. 150 during a stay in hospital. This patient (woman) and her older brother presented no clinical symptoms, whereas another brother, who also was older, presented deafness and previous arthritis of the knee. Two years later, No. 150 gave birth to a child which was admitted to the Ehlers Home (hospital for syphilitic children); its serum reaction was 3/— — — —, and later —/— — — —, without any treatment; so this was no instance of syphilis in the third generation.

Nos. 195 + 160 + 230: The lesion was first diagnosed in No. 195, a boy of 12 years who presented *parenchymatous keratitis, bilateral hydrarthrosis of the knee and Hutchinson teeth*. The two other sibs — a girl of 14 years and a boy of 8 years — presented no clinical symptoms, but both gave a very strong serum reaction. At the same time, syphilis was diagnosed in the mother, and the information was obtained that the father had had syphilis 22 years before.

Nos. 200 + 215 + 234: In these cases there was a positive family history, with syphilis in both parents. The disease was diagnosed first in No. 200 — a girl, 14 years old — when she was admitted to the Brejninge Institute for Mental Defectives; in addition she presented rhinitis and cervical adenitis. The two other sibs — a girl of 12 and a boy of 10 years presented *Hutchinson teeth*, besides a strong serum reaction.

Nos. 224 + 245 + 365: Syphilis was diagnosed first in No. 224 — a girl, 10 years old — who was hospitalized for deafness. A brother, 3 years younger, presented no clinical symptoms, but a strong serum reaction. Syphilis was ascertained in the mother at the same time; and one month later she gave birth to a boy with congenital syphilis (*rhagades at the mouth, papules on the face and round the anus, serum reaction 3/—*).

Nos. 251 + 259 + 390: Syphilis was diagnosed first in No. 251 — a girl, 8 years old — who had had *coryza* ever since birth. A

brother, 2 years old, and a sister, 7 months old, presented no clinical symptoms, but both gave a positive serum reaction. The father showed negative WR. Syphilis in the mother was diagnosed two weeks before it was diagnosed in No. 251. In addition, there were 3 older sibs who all gave — WR.

Nos. 356 + 278 + 264: In this family there were several other children besides the 3 included in the present material. The 2 oldest children were well, with — WR. Nos. 3, 4 and 5 had died respectively at the age of 3 years (from an accident), 5 weeks («heart failure») and 6 months. Nos. 6 and 7 belong to our material. No. 8 was well; No. 9 had died at the age of 7 months; No. 10 belongs to this material; and No. 11 was well. The father gave repeatedly — WR. Syphilis in the mother was ascertained in 1923, two years before the birth of the first child. Among the children, syphilis was ascertained first in No. 356 — a girl, 6 months old — with coryza and rash; a sister, 4 years old, presented no clinical symptoms, while a brother, 5 years old, presented a suggestion of saddle-nose, dental deformity and a hysterical habitus; both of these children gave a strong serum reaction.

In conclusion, mention is to be made of a family in which no less than 8 children had unquestionable congenital syphilis, while 2 other sibs were under observation for this affection. The family resided in Jutland, and syphilis in the mother was ascertained 4 years before the birth of the oldest child. Nothing is known about treatment of the mother. In the children syphilis was ascertained first in a girl, 7 months old, who was admitted to a hospital where she died of bronchopneumonia; she gave a very strong serum reaction, but otherwise she presented no definite clinical sign of congenital syphilis. The mother was then examined and found to have cerebrospinal syphilis. After this the other children were examined; all 7 gave a strong serum reaction but presented strikingly few clinical symptoms. Four of them were completely free from clinical symptoms; one girl, 14 years old, had Hutchinson teeth; another girl, 11 years old, presented a high palate and Hutchinson teeth, and the same features were found in a girl of 9 years.

The two children under observation were born in 1937 and 1940, i. e., after the mother again has been under antisyphilitic treatment, the children have only had weak and rapidly decreasing serum reactions, and presumably they are now to be looked upon as healthy.

Syphilis in the Third Generation.

The appearance of syphilis in the 3^d generation is a problem which always has involved rather considerable difficulties, as it will be difficult to find cases which meet the requirements that must be insisted

upon if they are to be recognized as actually representing syphilis in the 3' generation. In order to be able with certainty to assert that a patient is suffering from syphilis in the 3' generation, we have set up the following requirements:

1. It must be proved conclusively that the patient is suffering from congenital syphilis.
2. The mother must be suffering from congenital syphilis; the possibility of acquired syphilis must be ruled out.
3. The father must be perfectly healthy, *i. e.*, free from acquired and congenital syphilis.
4. In cases implying the least doubt the maternal syphilis being congenital, the presence of a syphilitic affection in the mother's mother must be demonstrable.

Other authors — *e. g.*, Finger, Haldimann and Dennie & Pakula — insist upon stricter requirements, demanding especially that in every case there must be positive data concerning the presence of syphilis in the first generation. In practice these data are often very difficult to obtain.

In 1936 Haldimann went through the literature and found a total of 12 patients with eye symptoms, in whom the affection presumably involved syphilis in the 3' generation. Of these patients 4 had to be ruled out because they did not meet the stipulated requirements; of the remaining 8 patients 7 had parenchymatous keratitis, 1 chorio-retinitis. Bergmeister and Manchot have each reported one case. In Bergmeister's case the patient presented parenchymatous keratitis together with a positive Wassermann reaction. The mother of this patient had bilateral parenchymatous keratitis, and her father was suffering from dementia paralytica. But information was lacking about the maternal grandmother and about the Wassermann reaction in the mother and father. So, in the stricter sense, this case was not absolutely certain. In Manchot's case the maternal grandfather had dementia paralytica and the grandmother tabes dorsalis, the mother presented no definite sign of congenital syphilis, and the patient had parenchymatous keratitis together with a strongly positive serum reaction. But information about the father was lacking.

In their monograph on congenital syphilis, Dennie & Pakula in 1940 took up the question anew. They examined altogether 14 families, in all of which the mother presented unquestionable signs of congenital syphilis. In these 14 mothers the clinical symptoms were: parenchymatous keratitis in 4 cases, disseminated chorioiditis in 2 cases, Hutchinson's triad in 2 cases, Hutchinson teeth in 4 cases (combined with bone syphilis in 2), syphilitic arthrosis of the knee in 1 case, and tabes dorsalis at the age of 30 in 1 case. The Wassermann reaction was positive in all the cases but one (syphilitic arthrosis of the knee in the mother, syphilis of the liver in the maternal grandmother, and Hutchinson's triad in a maternal aunt). The information

about the *fathers* was very defective: in 9 of the 14 cases the father was not seen at all, in 3 cases he gave a negative Wassermann reaction, in 1 case he refused to have a sample of his blood withdrawn, and in 1 case the patient was born out of wedlock. Likewise, the information about the maternal *grandmothers* was very incomplete: no information whatever was obtained in 7 cases, the Wassermann reaction was positive in 5 cases and negative in 1 case, and in 1 case the birth of the patient's mother was stated to have been preceded by 5 abortions. In addition there were some scattered data on the presence of syphilis in some of the sibs. The 14 mothers had altogether 22 children: 8 were symptom-free, serologically as well as clinically; 2 showed a positive reaction in the blood (*1 symptom-free patient*, 1 with a joint lesion), and 3 others showed a positive reaction in the spinal fluid (all three with cerebrospinal syphilis); on the remaining 9 seronegative children the following diagnoses were made: optic neuritis (3), syphilitic nodules in the vulva (2), *spastic hemiparesis* (1), bone syphilis (1), chorioiditis (1), and typical congenital syphilis (1).

After the strictest theoretical demands, Dennie & Pakula's material includes but one case of syphilis in the 3' generation (father seronegative; one child seropositive but symptom-free, another child seronegative with *spastic hemiparesis* — the two cases above in italics). The two other seronegative fathers have healthy children. — As the congenital syphilis in the mothers was unquestionable and apparently untreated, however, we find it safe to reckon that Dennie & Pakula have described the occurrence of syphilis in the 3' generation in 8 families with a total of 14 children, although the seronegativity of some of the children is a striking observation.

Investigations of similar character as the above, aimed to ascertain the fate of the children born of mothers with unquestionable congenital syphilis have been reported by several authors. Thus Igersheimer found the Wassermann reaction negative in 12 children born of mothers with parenchymatous keratitis; and the same applies to 44 of 45 children examined by Sidler-Huguenin.

In a material reported by Dalsgaard-Nielsen, a total of 90 children were born of 52 married mothers who had congenital syphilis. Of these children 4 died in infancy, 4 were weakly, 2 had epileptic convulsions and 80 were claimed to be perfectly well. Wassermann test had been performed only on 7 of these children; it turned out negative in every instance.

In our material we found 4 families in which we thought it was a question of syphilis in the 3' generation. In Table 17 a schematic survey is given of the 4 cases, together with 3 additional instances of this condition.

From Table 17 it will be noticed that the cases do not fulfil the

Table II.

Instance of Probable Syphilis in the 3^d Generation;

Pt. No.	Sex, age, year of birth	Patient's symptoms	Patient's serum reaction	Mother	Father	Mother's mother
A 72	Male, 22 years, b. 1913	Dermatitis. Parenchymatous keratitis (at age of 7—10 years) Periostitis. Dental deform.	1935 W/K: 2/2	Born 1888 1939, W/K: 7/6 Parenchymatous keratitis (several attacks)	Born 1881 +WR. Optic atrophy. Tabes. Syphilitic aortitis.	No information
B 166	Male, 16 years, b. 1922	Dental deformity	1939 W/K: 8/9	Born 1903 1939, W/K: 9/9	Born 1901 February 1926, +WR (discovered when he was pointed out as source of infection) Dec. 1926: —WR	Born 1873 1938, W/K: Enlargement of cervical glands
C 287	Female, 4 years, b. 1934	Parenchymatous keratitis. Hydrarthrosis of the knee	1939 WR: 12 KR: unreadable	Born 1912 W/K: 7/5 Deafness. Syphilitic neurolabyrinthopathy? Congenital syphilis	—WR	Deaf.
D 389	Female, 0 years, b. 12/4/39	Died 3 days old. Syphilitic cirrhosis of liver. Asphyxia. Trachea membranacea. Partial atelectasis of both lungs	1939 —WR umbilical cord blood	Born 1911 1926: +WR Ulceration of palate and nasopharynx. Congen. syph. (Dep. H). Feeling well. Last cure before delivery, October 1938	Born 1904 Congen. syph. diagnosed in 1917. Since 1923, WR negative	No information

17.

4 Cases in the Present Material and 3 Additional Cases.

Mother's mother	Mother's father	Sibs of patient	Sibs of mother	Critical remarks
No information	No information	6—7 will not submit to examination		Father acquired syphilis. Mother's mother: no information.
Born 1873 1938, W/K: 8/9 Enlargement of cervical glands	Born 1873. 1925: +WR 0, 60, 100. Ulceration on lip; no treatment. 1939, W/K: 11/15	1, female —WR	Male, b. 1895; —WR; 8 children, all well. Female, b. 1897. In 1939 +WR; in 1923 she had syph. child who gave +WR in 1924: 0, 0, 0, 60, 100. Child in Welander Home, given 61 mercurial inunctions before adm.; WR then neg. Mother claims her WR was neg. several times prior to 1929. In 1939, W/K: 5/4. Father: no information about WR and syphilis. Male, b. 1901; —WR. Female, b. 1905; —WR. Female, b. 1910; +WR (1939)	Mother symptom-free, but her sister shows +WR. Syphilis in father undoubtedly acquired after the birth of the patient.
Deafness	No information	Deafness		Mother's mother: No information about her WR.
No information	No information	1, born 1905 Observation for syphilis. No further information		Father had congenital syphilis but is well treated. Mother's mother: No information.

Table 17.

Pt. No.	Sex, age, year of birth.	Patient's symptoms	Patient's serum reaction	Mother	Father
E	Male, 7 years, b. 1933	»Boils« in infancy. 1937: Otitis. 1940: Hepatitis. Now: Saddle-nose, rachitic skull, marked chorioretinitis diss. Referred to Institute for the Blind	1940 W/K: 10/10	Born 1915 1917, 1½ years old: +WR (0, 0, 100) Papules at anus. Antisyphilitic treatment; only control in 4 years. 1940, W/K: 1/4. Disturbance of speech. Loss of memory. Somewhat demented, perhaps beginning dementia paralytica	Born 1911 1940, W/K: —/—
F	Male, 7 months, b. 1940	Syphilitic skin lesion	1941 W/K: 14/≥12	Born 1919 1941, W/K: 9/9 symptom-free	Born 1918 1940, W/K: —/—
G	Male, 12 years, b. 1929	Symptom-free	1942 W/K: 5/3	Born 1907 1942, W/K: 16/10 symptom-free	Born ? W/K: —/—

conditions required for an unqualified statement about syphilis in the 3' generation.

The most certain case is that of patient No. 166 — Table 17, B. This involves a thoroughly infected family; and really it seems strange that all the cases in this family were not disclosed till 1939, as a possibility of this had offered itself twice before: the first time in 1924, when congenital syphilis was ascertained in a child born in 1923 of a woman who was born in 1897; but nothing was done about the mother as she claimed to be well. The child was admitted to the Welander Home, where he soon became negative. The second time was in 1925 when the maternal grandfather was hospitalized for ulceration of the lip; he gave + WR, and microscopy of the ulcer showed chronic inflammation, but no further examination of the family was carried out. In 1938 the maternal grandmother was hospitalized for enlargement of the cervical lymph glands; also she gave + WR (8/9). On this occasion WR was repeated on the grandfather who now showed a stronger positive reaction (11/15); in addition, a test was performed on the mother (born 1903) with + WR (9/9), on 2 sisters (born 1897 and 1910) also with + WR; besides

(Continued)

Mother's mother	Mother's father	Sibs of patient	Sibs of mother	Critical remarks
Born 1869. 1917, acquired syphilis; +WR 0, 60, 100; antisyphilitic treatm.	No information	Female, b. 1938. 1940, W/K: 12/14; saddle-nose, protruding brow, granular chorioid configuration; admitted to Welander Home. Female, b. 1936. 1940, W/K: —/—	2 sibs, born 1908 and 1909, both giving —WR.	Mother had perhaps acquired syphilis, but congenital syphilis is more likely.
Born 1900. 1940, W/K: 8/8; symptom-free	Born 1897. 1939, W/K: 6/5 (hospitalized for sciatica)		Female, b. 1931. W/K: 10/≥1 symptom-free	Symptoms of congen. syph. absent in the mother, but her sister shows +WR.
Born 1883. 1939, W/K: 19/14. Aortitis periostitis	Information about acquired syphilis	Male, b. 1935 1940, W/K: 10/7; symptom-free	4 sibs, free from syphilis (—WR)	Symptoms absent in the mother.

in 3 other sibs (male, born 1901, male, born 1895; and female, born 1905) who showed —WR. The father of the patient, born 1901, was found to give +WR in February 1926, when two women had reported him as source of infection; he presented no sign of congenital syphilis, and after antisyphilitic treatment he became seronegative already in December 1926, on which account we take his affection to have been a recent acquired syphilis, contracted after the birth of the child.

The three other cases are somewhat less clear-cut, primarily because information about the grandparents is lacking in all three instances. Patients Nos. 72 and 287 (Table 17, A and C) presented pronounced clinical symptoms of congenital syphilis, and both gave a positive serum reaction. A fairly strong positive serum reaction was obtained in both mothers, and one mother presented parenchymatous keratitis, while the other showed bilateral syphilitic neurolabyrinthopathy, so that the diagnosis congenital syphilis was made in the respective special clinics; but, as mentioned in Art. I, p. 267, we have not considered it fully established that the deaf mother had congenital syphilis. In No. 287 (Case C) there is definite information about —WR

in the father, but in No. 72 (Case A) the father showed both clinical and serological evidence of syphilis.

The fourth case, No. 389 (Table 17, D) was that of a girl, 3 days old, who died. Autopsy revealed cirrhosis of the liver from congenital syphilis; blood from the umbilical cord showed — WR. In the mother + WR was recorded in 1926 together with ulceration of the palate and nasopharynx, when the diagnosis congenital syphilis was made by the special clinic. The father gave a history of congenital syphilis that was diagnosed in 1917 and was treated effectively, so that he has been seronegative since 1923.

The last 3 cases are not quite certain, it has to be admitted, but still we think that our material possibly includes 5 cases of syphilis in the 3' generation, 2 of these in one family.

In 1940—42 we have found 3 additional families who likewise, perhaps illustrate the occurrence of syphilis in the 3' generation.

Case E.

In 1940 the mother (born 1915) brought her child (born 1933) to an eye clinic in Copenhagen — on account of the poor vision of the child. The mother showed WR = 1, KR = 4. Subsequent examination of the mother in the neurological clinic — because of impairment of her memory — showed lowered capacity for inculcation, and disturbance of speech but — WR in the spinal fluid. The diagnosis was made: Observation for dementia paralytica?

The child showed WR = KR = 10, and gave the following history: »Boils on the trunk« in infancy; otitis in 1937; hepatitis, slight degree, in 1940 (WR not performed). In the eye clinic the following features were found: Saddle-nose, rachitis skull, pupils and cornea normal, very pronounced bilateral disseminated chorioretinitis. The patient was referred to the Institute for the Blind.

The sibs of the patient were: girl (born 1936) with — WR, and a girl (born 1938) with WR = 12, KR = 14, saddle-nose and protruding brow; eye examination showed merely a little granular choroid configuration.

As to the family history the following data were obtained; father (born 1911) well, with — WR in 1940. Mother's mother (born 1869) was treated for syphilis in 1917, in a country hospital. Two sibs of the mother (born 1908 and 1909) showed — WR in 1917, at the same time as the mother (then 1½ years old) showed WR 0,0,100 and papules at the anus; she was given calomel treatment and at least 50 mercurial inunctions of 1 gr.; on the last control examination, in 1921 she showed — WR.

The serious eye lesion in the oldest child ought to have been avoided if the treatment of the mother and/or the control examination had been carried through better (repetition of the treatment in 1932—33). The possibility cannot be excluded that the mother's lesion

(papules at the age of $1\frac{1}{2}$ years) may have been acquired syphilis, but this is not particularly probable as the only symptoms in the grandmother were + WR and inguinal adenitis.

Case F.

In 1941 a general practitioner in Jutland diagnosed a syphilitic skin lesion in a boy of 7 months (born 1940). His mother (born 1919) presented no symptom of syphilis other than + WR. Both the mother's sister (born 1931) and her mother (born 1900) showed + WR. The mother's father (born 1897) had latent syphilis which was diagnosed in 1939 during his hospitalization for sciatica. The father of the child (born 1918) showed — WR in 1941.

The neglect of examination of this family in 1939 brought about that the grandchild (born 1940) had congenital syphilis.

Case G.

In the third family the diagnosis of the lesion was made in 1939—42. In 1939 the mother's mother (born 1883) was hospitalized for aortitis and periostitis, and showed WR = 19, KR = 14. In 1942 + WR but no symptoms were found in the mother (born 1907) and in 2 brothers (born 1929 and 1935). The mother's mother states that she was infected by her husband in 1905 (of her 4 children, only No. 2 had syphilis. As the father of the boys showed — WR this case appears fully established.

If examination of the family had been carried through in 1939 the boys would have been given treatment about $1\frac{1}{2}$ years earlier and thus have had a chance of a more rapid recovery (cf. behavior of the serum reaction under treatment).

Summary.

A review is given of the data concerning syphilis in the mothers, in particular the point of time for the diagnosis.

In 71 of 178 cases the maternal syphilis was not diagnosed till syphilis had been demonstrated in the child.

The groups of sibs in the present material are reviewed, and mention is made of sibs outside the material.

The question about syphilis in the 3' generation is discussed on the basis of the literature.

Data are given concerning 4 cases taken from the present material, together with 3 cases from 1940—42, which perhaps represent syphilis in the 3' generation. The 7 families include altogether 10 children with congenital syphilis.

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GRAPHICAL EVALUATION OF THE DISTRIBUTION OF SMALL EXPERIMENTAL SERIES

By *Johs. Ipsen* and *N. K. Jerne*.

(Received for publication Jan. 18th 1944).

In many cases data from clinical, toxicological and other biological investigations are in the form of quantitative measurements which are arranged in groups according to the conditions under which the measured quantities are present or produced. A statistical treatment of such data is frequently required, *e.g.*, in order to judge the certainty of the difference between the mean reactions of the individual groups, or to describe a relation between mean value and the degree of the action to which the groups have been subjected, etc. The biometric investigations of the last decades have made highly developed methods available for this purpose, but many of these statistical tests have as a necessary qualification that the numerical quantities in question must be distributed according to the normal — the so-called Gaussian — distribution curve.

Statistical analyses of this kind of experimental series should begin with an investigation of whether the values in each group may be assumed to show a normal distribution. For this purpose, as the present paper will demonstrate, graphical methods are available which are not only practicable, but also have the advantage of giving an immediate idea of the mean value and standard deviation of a normal distribution.

Should it be found that the numerical values in question are not normally distributed, several methods may be chosen of which two will be mentioned here; the second of these two is the most attractive from the point of view of the biologist. The first method follows that developed by *Karl Pearson*: To find the type of distribution to which the material fits. Pearson's distribution types require the calculation of several parameters of higher order than the two — mean and standard deviation — which completely describe the normal

distribution. Even though it may be possible in this way to get to the formula for many asymmetric distributions the work is extremely complicated, and the constants that enter into the formula are frequently of no biological interest. Moreover, these calculations require a larger material of observations than most biologists have at their disposal.

The other procedure consists in finding a function of the metric values which is normally distributed. Even though it may be assumed that the phenomena observed are subject to chance variation it is in no way certain that the metric values which for practical reasons have been chosen to describe the intensity of the phenomenon are normally distributed. *R. Pearl*, in »Introduction to Medical Biometry and Statistics«, cites as an example of asymmetric distribution the pulse frequency measured in 924 convicts, and shows how this distribution through a long series of mathematical operations may be fitted to one of Pearson's distribution types. If instead of the pulse frequency per minute as unit he had chosen *the reciprocal value* of this quantity — the duration of the pulse beat — he would have obtained normally distributed values, where the calculation is confined to the finding of mean and standard deviation.

Serological potency determinations expressed in titre or units per cc are not normally distributed, but the *logarithms* of these values usually are.

The graphical method described in the following makes it possible to judge rapidly whether functions of the observation, x , as for example $1/x$, $\log x$, x^a , etc., are normally distributed, whereupon the statistical investigations are carried out on that function of x which proves most suitable for the purpose. Any *proof* that just this particular function is normally distributed is not furnished by the graphical method, no more than by the more complicated arithmetical method described in various handbooks. By these methods one can judge whether the distribution departs so grossly from the normal that it is no longer permissible to apply the tests based on such normal distribution.

The Histogram.

The classical way of representing a distribution is by means of the so-called histogram where the values observed are plotted as abscissae while the ordinates are the number of observations which have the magnitude of the abscissae value. In order to find a suitable ordinate size it is usual to divide the axis of abscissae into equidistant groups, and the distribution is then recorded as a series of rectangles, the base of each being the abscissae graduation and the height the frequency of observations within each abscissae interval.

The larger the number of observations, the narrower one may choose the intervals. In the case of a very large, normally distributed experimental series, where the intervals can be made »infinitesimally« small, the top of the rectangles will form a smooth curve of the characteristic symmetrical bell shape.

If one is to prepare a histogram for a distribution, and compare its shape with that for a normal distribution, it is recommended first to calculate the mean, \bar{x} , and standard deviation, s , for the distribution and then plot the abscissae values \bar{x} , $\bar{x} \pm a \cdot s$, $\bar{x} \pm 2a \cdot s$, etc. ($a = 1, \frac{1}{2}, \frac{1}{3}$ or $\frac{1}{5}$, all according to the number of observations included in the distribution).

Example: Prigge gives the following death times, in days, for 20 guinea-pigs infected subcutaneously with 100 mg of tubercle bacillae: 10, 10, 20, 20, 25, 32, 33, 35, 38, 39, 39, 46, 48, 49, 49, 50, 52, 52, 54, 72.
 $\bar{x} = 38.65$ $s = 15.9$ The unit of grouping is put equal to 1s.

Table 1.

Group limits given in		Number of observations in the groups	Expected obs. $\bar{x} + y \cdot s$	Expected obs. in the groups	Expected number in the groups
death time	symbols.				
(1)	(1)	(2)	(3)	(4)	(5)
-9.1	$\bar{x} - 3s$		0.15%		
6.8	$\bar{x} - 2s$	0	2.27%	2.1%	0.5
22.7	$\bar{x} - 1s$	4	15.86%	13.6%	2.7
38.65	\bar{x}	5	50.00%	34.1%	6.8
54.8	$\bar{x} + 1s$	10	84.14%	34.1%	6.8
70.5	$\bar{x} + 2s$	0	97.73%	13.6%	2.7
86.4	$\bar{x} + 3s$	1	90.85%	2.1%	0.5

Table 1, column 1 gives the group limits together with the corresponding symbols. The ordinates in the histogram are now obtained by counting the number of observations that fall within these limits (column 2), as shown in fig. 1 by fully drawn lines. For comparison a histogram is constructed for a normal distribution with the same mean, standard deviation and unit of grouping for the 20 observations. Most statistical handbooks contain tables which show how many per cent of the individual observations in a normal distribution are $= \bar{x} + y \cdot s$,*) where y is the deviate from the mean in units of

*) The tables are so arranged that one enters the table under a percentage (P) and finds the corresponding y .

$$\text{Mathematically P is equal to } 100 \frac{1}{\sqrt{2\pi}} \int_{-\infty}^y e^{-\frac{t^2}{2}} dt$$

standard deviation. The percentages corresponding to $y = -3, -2, \dots, +3$ are recorded in column 3. It will be seen that for $y = 0$ we find 50 %, which agrees with the circumstance that in an ideal normal distribution the middle item (the median) is just larger than one-half of the observations.

Next the expected percentage in each group (column 4) is found as the difference between two consecutive values in column 3. Finally, the expected absolute number in the groups is calculated as percentage (given in column 4) of the total number 20 (column 5). With the

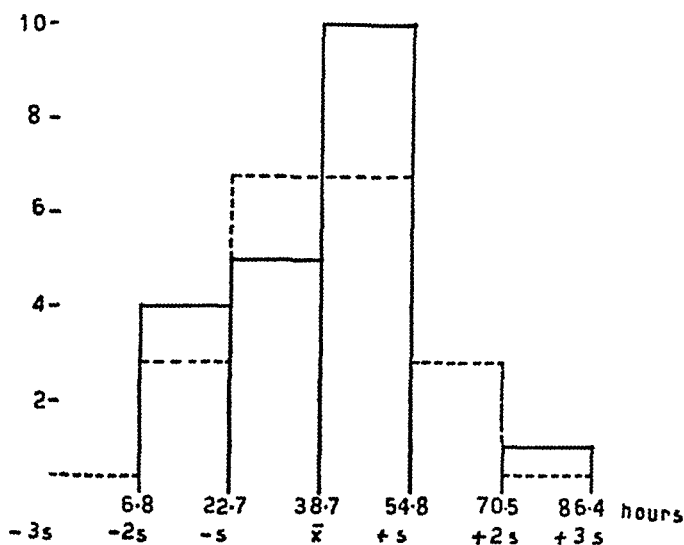


Fig. 1.

items in column 5 as the height and the abscissae in column 1 as the base, a histogram is now prepared corresponding to this ideal normal distribution of 20 observations, as shown in fig. 1 (dotted line).

It is difficult, however, to judge objectively whether a histogram like this one deviates significantly from that of the normal distribution. The number of observations is too small, and the factors which must enter into the judgement too many to make a satisfactory answer possible. Arithmetically it is possible by means of *Pearson's* χ^2 -test to investigate whether the observed and the expected numbers in the groups deviate significantly, but, as is well known, this test must not be applied to groups the expected number of which is less than 5, and it is therefore necessary to combine adjoining groups until the expected number exceeds 5. In this case there would thus only be 2 groups, namely 1 above and 1 below the mean, and the effectivity of the χ^2 -test is then considerably weakened.

However good the information one may obtain from a histogram

when it is a question of large materials, the method is less suited in the case of numerous small experimental series of the kind that the biologist so frequently deals with.

The Probit Method.

Another more lucid graphical test has as its first step that the observations are ranked according to magnitude. They are then plotted directly as abscissae, while the ordinates are quantities which are dependent on the place of the observations in the series and which are so determined that the points with these coordinates will *fall on a straight line* if the distribution is normal. These ordinates are the variable which above was designated by y and which may be defined as the abscissa in a normal distribution with unit standard deviation and mean value 0. If we know how many per cent of the observations are smaller than or equal to an observed value, we are able to determine the deviate (y) corresponding to this value. In the above example dealing with the death time of tuberculous guinea-pigs the first 2 values are ≤ 10 , which gives the percentage $\frac{2}{20} \cdot 100 = 10\%$. y is then found in the above mentioned table*) by looking under 10 %. The cumulative percentage 95 corresponds to the second highest value, 54 hours, and the percentage $\frac{n-r+1}{n} \cdot 100$ corresponds to the r 'th highest value.

Table 2.

Death time	Cumulative number	Percentage	y	Probit ($y + 5$)
10	2	10 %	-1.28	3.72
20	4	20 %	-0.84	4.16
25	5	25 %	-0.67	4.33
32	6	30 %	-0.52	4.48
33	7	35 %	-0.39	4.61
35	8	40 %	-0.25	4.75
38	9	45 %	-0.13	4.87
39	11	55 %	+ 0.13	5.13
46	12	60 %	0.25	5.25
48	13	65 %	0.39	5.39
49	15	75 %	0.67	5.67
50	16	80 %	0.84	5.84
52	18	90 %	1.28	6.28
54	19	95 %	1.64	6.64
72	20	100 %	(∞)	(∞)

Table 2 illustrates this procedure, and the graphical representation is made by plotting y against the death time. *Gaddum* calls the

*) e. g., *Fisher and Yates* »Statistical Tables«, Table IX.

quantity y the »normal equivalent deviation«. It is tabulated in its most practical form by *Bliss* (1) who calls the quantity »probit« (probability unit) and adds 5 to avoid negative values. Hence the graphical method is in more recent literature known as the *probit method*.

As mentioned, the points with probits as ordinates and the observations as abscissae shall form a straight line, aside from chance variations, provided it is a question of normal distribution. In practice, however, the probit method involves certain systematic errors owing to the circumstance that the theory is transferred from a continuous distribution of an infinite number of elements to a limited number of observations. This causes a systematic displacement towards too high probits, which readily may be verified in the case of two values — the middle one and the highest. The median in an ideal normal distribution should correspond to the cumulative frequency 50 %. In a distribution of 20 quantities, 50 % should therefore correspond to a value between the 10th and the 11th highest in the series, but, as seen from table 2, the procedure described makes 50 % correspond to the 11th highest value, so that a percentage $100/2n$ too high is assigned to the mean by the graphical method. Owing to displacement, the highest value will get the percentage 100, hence no finite probit value can be assigned to it.

This bias can be avoided, according to *Bliss* (2), by basing each percentage upon the total number of observations smaller than the particular observation plus one-half of the one or more observations equal to the one in question. As will be shown later, this seemingly arbitrary procedure gives good approximation, but only if each observation occurs but once. All percentages will then be reduced by $100/2n$, and since the highest value now gets a percentage of less than 100 it is possible to assign a probit value to it.

Cavalli and *Magni* are of the opinion that *Bliss'* correction will eliminate half of the last observations, and propose instead of plotting y as a single point to picture the region which is bounded by ± 1 times the standard deviation of y as a vertical line segment parallel to the axis of ordinates. Inasmuch as the observation 100 % has a lower limit of error, the last observation too can be incorporated in the curve. The »Mutungsbereich« proposed by *Prigge* is used as limits of error. *Cavalli* and *Magni*, however, do not avoid in the procedure a systematic displacement of the values, and this circumstance is especially unfortunate since the probit method in the paper in question has for its very purpose a calculation of the median.

The probit method is used to a constantly increasing degree in the statistical treatment of experimental data. All authors have been aware that the method yields but a rough estimate. There is nothing that tells against the use of a practical method, even though it is encumbered with error, provided only these errors are not systematical.

It is necessary to realize which systematic errors a method involves and to eliminate these, even though they may seem to be of a slight order of magnitude. In the following we shall describe an attempt to provide an exact solution of the question involved, and then compare the result with the probit method.

Rankits.

We assume that some kind of observations are normally distributed about a »true« mean value ξ , and that n such observations are made. These n values with their chance variation are now ranked according to magnitude. If we then repeat n measurements several times and rank the data it will be found that the r 'th highest value ($r = 1, 2, \dots n$) will vary about a value which has a given distance from ξ , positive if r is $\leq \frac{1}{2} n$ and negative if r is $> \frac{1}{2} n$. We will now find this distance u in a normal distribution having mean value 0 and unit standard deviation.

Since u is a given abscissae value in the normal distribution (fig. 2) the probability p of a random-picked value being less than u is expressed by the part which the area to the left of an ordinate through u constitutes of the whole area. This can be read from the probit table, thus

$$\text{probit } (p) = u + 5.$$

The probability of this value being higher than u is $1-p$. Moreover, the probability of $n-a$ values being smaller than u and the other a values greater than u is equal to

$$\frac{n!}{(n-a)! a!} p^{(n-a)} (1-p)^a$$

The probability of the highest of the n values ($r=1$) being smaller

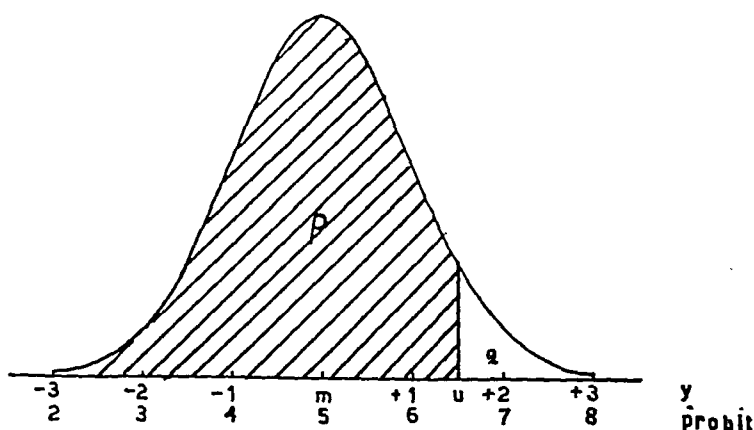


Fig. 2.

than u is equal to the probability of all values being smaller than u , i. e., $a = 0 = r-1$,

$$P(n, 1) = p^n$$

The probability of the second highest value ($r = 2$) being smaller than u is equal to the sum of the probabilities of all values being smaller than u and of $(n-1)$ values being smaller than u

$$P(n, r) = \sum_{a=0}^{n=r-1} \frac{n!}{(n-a)!a!} p^{(n-a)} (1-p)^a$$

By putting the probability $P(n, r)$ equal to a certain value, e. g., 0.1586 and 0.8414, it is possible, for given n and r , from the above expression to find the corresponding values $p_{.16}$ and $p_{.84}$, and from the probit table $u_{.16}$ and $u_{.84}$ corresponding to these values. These two values of u are the limits within which the r 'th highest of n values with 68.28 % probability will fall, thus a region which is of the same magnitude as that lying within $(\bar{x} + 1)$ and $(\bar{x} - 1)$ times the standard deviation in a normal distribution. Between these 2 values is the mean of the distribution of the r 'th highest of n values. We designate it by \bar{u} and calculate

$$\bar{u} = 1/2 (u_{.16} + u_{.84})$$

Example: Calculation of \bar{u} for the highest of 10 values ($r = 1$).

$$0.1586 = (p_{.16})^{10}; p_{.16} = 0.8319; \text{probit } (83.19 \%) = 5.9617;$$

$$u_{.16} = 0.962.$$

$$0.8414 = (p_{.84})^{10}; p_{.84} = 0.9828; \text{probit } (98.28 \%) = 7.1165;$$

$$u_{.84} = 2.117.$$

$$\bar{u} = \frac{1}{2} (0.962 + 2.117) = 1.540.$$

Table 3.

Tables of rankits.

\bar{u} is the average deviation of the r 'th largest of n samples drawn from a normal distribution with unit standard deviation.

Table of \bar{u} .

	$n = 2$	3	4	5	6	7	8	9
r								
1	0.564	0.847	1.030	1.163	1.268	1.353	1.424	1.486
2		0.000	0.297	0.494	0.642	0.749	0.853	0.932
3				0.000	0.202	0.353	0.473	0.572
4						0.000	0.158	0.275
5								0.000

Table 3 (cont.)

	n = 10	11	12	13	14	15	16	17
r								
1	1.540	1.587	1.630	1.669	1.704	1.737	1.767	1.795
2	1.001	1.062	1.116	1.164	1.208	1.249	1.285	1.319
3	0.656	0.728	0.793	0.850	0.901	0.948	0.991	1.030
4	0.376	0.462	0.537	0.603	0.662	0.715	0.763	0.808
5	0.123	0.225	0.313	0.389	0.456	0.516	0.570	0.620
6		0.000	0.102	0.190	0.267	0.336	0.396	0.452
7				0.000	0.088	0.116	0.234	0.296
8						0.000	0.078	0.146
9								0.000

	n = 18	19	20	21	22	23	24	25
r								
1	1.821	1.845	1.868	1.889	1.910	1.930	1.948	1.966
2	1.350	1.380	1.407	1.434	1.459	1.482	1.504	1.524
3	1.066	1.100	1.131	1.161	1.188	1.215	1.240	1.263
4	0.849	0.886	0.921	0.954	0.985	1.014	1.041	1.067
5	0.665	0.707	0.746	0.782	0.816	0.848	0.877	0.905
6	0.502	0.548	0.589	0.630	0.667	0.702	0.734	0.764
7	0.351	0.403	0.448	0.492	0.532	0.569	0.604	0.637
8	0.208	0.264	0.315	0.362	0.406	0.446	0.484	0.518
9	0.068	0.131	0.187	0.239	0.286	0.330	0.370	0.409
10		0.000	0.064	0.119	0.170	0.218	0.262	0.303
11				0.000	0.057	0.108	0.156	0.200
12						0.000	0.052	0.100
13								0.000

Table of $u_{.16}$ (on the left hand) and $u_{.84}$ (on the right hand).

$u_{.16}$ and $u_{.84}$ are the lower and the upper limits of the deviation of the r 'th largest sample with a probability corresponding to the limits ± 1 time the standard deviation of a normal distribution.

	n =	2	3	4	5
r					
1		— 0.258 1.387	0.104 1.590	0.335 1.725	0.502 1.825
2			— 0.668 0.668	— 0.302 0.896	— 0.061 1.052
3					— 0.335 0.535

	n =	6	7	8	9
r					
1		0.631 1.905	0.735 1.970	0.822 2.026	0.897 2.075
2		0.115 1.169	0.253 1.245	0.365 1.340	0.460 1.404
3		— 0.294 0.697	— 0.116 0.821	0.026 0.920	0.142 1.002
4			— 0.458 0.458	— 0.279 0.584	— 0.138 0.687
5					— 0.407 0.407

	n =	10	11	12	13
r					
1		0.962 2.117	1.019 2.155	1.070 2.189	1.117 2.220
2		0.541 1.461	0.612 1.512	0.675 1.556	0.731 1.597
3		0.239 1.073	0.323 1.134	0.397 1.189	0.462 1.237
4		— 0.021 0.772	0.078 0.846	0.164 0.909	0.239 0.967
5		— 0.265 0.511	— 0.148 0.598	— 0.048 0.673	— 0.038 0.739
6			— 0.370 0.370	— 0.253 0.456	— 0.153 0.532
7					— 0.341 0.341

Table 3 (cont.)

n =		14		15		16		17	
r									
1		1.159	2.249	1.198	2.275	1.234	2.300	1.267	2.322
2		0.782	1.634	0.829	1.668	0.871	1.699	0.910	1.728
3		0.521	1.281	0.574	1.321	0.623	1.358	0.668	1.392
4		0.306	1.017	0.366	1.063	0.421	1.105	0.471	1.144
5		0.114	0.797	0.182	0.850	0.243	0.896	0.299	0.940
6		-0.066	0.600	0.010	0.661	0.079	0.713	0.141	0.762
7		-0.242	0.418	-0.155	0.487	-0.077	0.545	-0.009	0.600
8				-0.318	0.318	-0.232	0.387	-0.155	0.447
9								-0.300	0.300

n =		18		19		20		21	
r									
1		1.298	2.344	1.326	2.363	1.354	2.382	1.378	2.400
2		0.946	1.754	0.981	1.779	1.012	1.802	1.042	1.825
3		0.709	1.423	0.747	1.452	0.782	1.479	0.816	1.506
4		0.517	1.180	0.559	1.213	0.599	1.243	0.635	1.272
5		0.349	0.980	0.396	1.017	0.440	1.051	0.480	1.083
6		0.197	0.806	0.248	0.847	0.296	0.881	0.340	0.920
7		0.053	0.649	0.112	0.694	0.161	0.735	0.209	0.774
8		-0.086	0.502	-0.024	0.551	0.033	0.597	0.085	0.639
9		-0.223	0.358	-0.154	0.416	-0.092	0.466	-0.035	0.512
10				-0.282	0.282	-0.215	0.343	-0.153	0.390
11								-0.272	0.272

n =		22		23		24		25	
r									
1		1.403	2.417	1.426	2.433	1.448	2.448	1.468	2.463
2		1.070	1.847	1.098	1.866	1.122	1.885	1.146	1.903
3		0.847	1.529	0.877	1.552	0.905	1.574	0.931	1.595
4		0.670	1.299	0.703	1.325	0.733	1.349	0.762	1.372
5		0.518	1.113	0.553	1.142	0.586	1.167	0.617	1.193
6		0.380	0.953	0.419	0.984	0.455	1.012	0.488	1.040
7		0.254	0.810	0.295	0.843	0.334	0.874	0.370	0.904
8		0.134	0.678	0.178	0.714	0.220	0.748	0.256	0.780
9		0.018	0.554	0.066	0.593	0.110	0.630	0.153	0.665
10		-0.096	0.436	-0.043	0.478	0.005	0.518	0.051	0.555
11		-0.208	0.321	-0.151	0.367	-0.099	0.410	-0.050	0.450
12				-0.259	0.259	-0.202	0.305	-0.149	0.348
13								-0.249	0.249

Table 3 records the values of u_{16} , u_{84} and \bar{u} for values of n ranging from 2 to 25, as well as the first half of the r -values. The value of \bar{u} for $(n-r+1)$ is found by giving the value \bar{u} for r opposite sign.

The values of \bar{u} are also tabulated by *Fisher* and *Yates* in »Statistical Tables«, table XX, for values ranging from 2 to 50. The above derivation is inspired by this table, but the application of the table for the purpose which is dealt with in the present paper is not men-

tioned by *Fisher* and *Yates* who merely introduce it with the following remarks:

»It is often necessary to draw statistical conclusions from data giving the order of a number of magnitudes, without knowledge of their quantitative values. Thus in tests of psychological preference subjects can often express preferences without being able to assign numerical values to the force with which the preference is felt. Not infrequently, also, an experimenter who possesses quantitative values may suspect that the metric used is unsuitable to the comparison he wishes to make, and prefer to draw conclusions only from the order of the magnitudes observed.

The analysis of such data is greatly facilitated by Table XX, which gives the average deviate of the r 'th largest of samples of n observations drawn from a normal distribution having unit variance. Symbolically this comes to

$$n_r = \int_{-\infty}^{\infty} \frac{n!}{(r-1)!(n-r)!} p^{n-r} q^{r-1} x z dx$$

where z is the ordinate of the normal curve, and p and q are the probabilities respectively falling short of and exceeding x .«

\bar{u} and *Fisher's* n_r have the same numerical value, but it is very troublesome from *Fisher's* expression to get an idea of the spreading about \bar{u} .

We propose the designation »rankit« for \bar{u} (unit of ranked data).

Comparison between probit and rankit.

It has been mentioned that the probit method gives a good approximation when *Bliss'* correction is applied. Actually, there is very little difference between rankits and probits, provided *Bliss'* correction is understood to mean that instead of calculating the cumulative frequency for the r 'th highest value as $\frac{n-r+1}{n} 100$ we use the corrected expression $\frac{n-r+1/2}{n} 100$.

Table 4 shows how large the deviation is for different values of n .

It will be seen that the probit values (minus 5) at the end of the series ($r=1, 2, 3$ and $r=n-2, n-1, n$) depart in the 2nd decimal from the rankit values and approximately the same in the case of $n=25$ and $n=100$. For $n=200$ and $r=1$ probit (minus 5) is 2.81 and rankit 2.75, difference 0.06. Between $r=4$ and $r=n-3$ the approximation is as good as one needs in practice. We may therefore very well use probits (minus 5) for values above 25, when we use the correction mentioned, and moreover correct by subtracting 0.07, 0.03 and 0.01 when r is 1, 2 and 3 respectively.

Example: $n=75, r=2$. Corrected percentage $\frac{73.5}{75} 100 = 98 \%$.

Probit (98) = 7.05. Rankit (75_2) = $7.05 - 5 - 0.03 = 2.02$.

Table 4.

r	n=25				n=100			
	% cor- rected*)	Probit —5	Rankit	Diff.	% cor- rected	Probit —5	Rankit	Diff.
1	98	2.05	1.97	0.08	99.5	2.58	2.51	0.07
2	94	1.55	1.52	0.03	98.5	2.17	2.15	0.02
3	90	1.28	1.26	0.02	97.5	1.96	1.95	0.01
4	86	1.08	1.07	0.01	96.5	1.81	1.80	0.01
5	82	0.92	0.91	0.01	95.5	1.70	1.69	0.01
6	78	0.77	0.76	0.01	94.5	1.60	1.59	0.01
7	74	0.64	0.64	0.00	93.5	1.51	1.51	0.00

Some applications of rankits.

1. The application of rankit in judging whether a series of data is normally distributed is a very simple process. After the data have been ranked, points are plotted in a coordinate system in such a way that for each point the abscissa is the observation or a function thereof and the ordinate the rankit determined by the rank number.

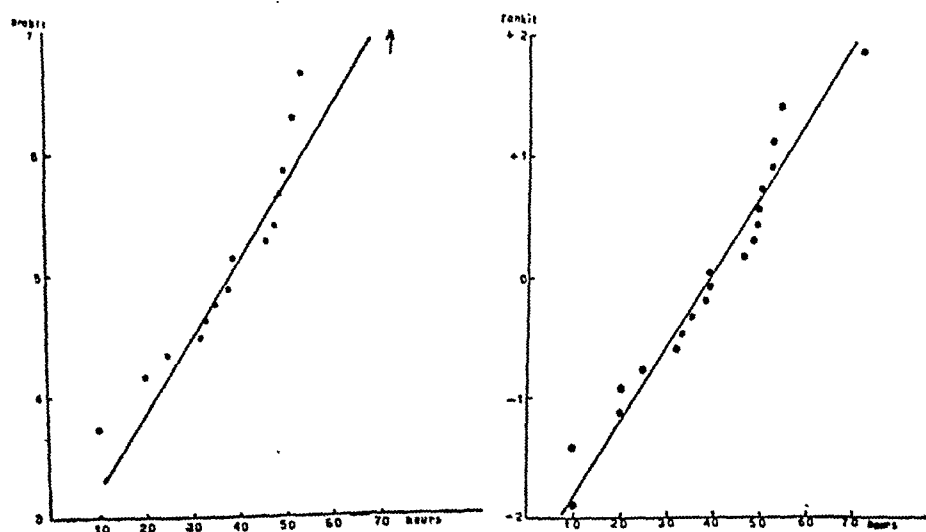


Fig. 3.

*)
$$= \frac{n-r+1/2}{n} \cdot 100$$

If the distribution is normal these points shall vary casually about a straight line. The mean of the distribution has then the ordinate 0 and the standard deviation is the reciprocal value of the coefficient of direction for the slope.

Fig. 3 shows a graphical representation of the distribution of death times for the 20 tuberculous guinea-pigs mentioned above. At the left the probit method is used without correction, corresponding to the data recorded in table 2, first and last column. At the right rankits are used as ordinates. The straight line is drawn through the calculated mean and with a slope that is determined by the calculated standard deviation. The probit method gives the impression of a systematic curvature, since the highest death time has no finite probit. This apparently systematic deviation is not found in the rankit diagram.

One of the most important reasons why it is of interest to investigate whether a series of observations is normally distributed is the desire to compare the series in the best possible way with series obtained from similar experiments. To let the result of the investigation of a single series decide as to the judgement of what function of the observation is normally distributed would be too arbitrary and uncertain. It must therefore be recommended to record all available experimental series in rankit curves in order to get a more adequate result.

In that respect it is especially important to undertake this investigation on groups with strongly varying means. It is not rare that a function apparently is normally distributed within a certain range of the observation, but that the examination of groups falling outside this range makes it necessary to look for a function that is more universally applicable.

The point is illustrated in fig. 4 which includes rankit curves from experiments on perfringens toxin injected in different doses into groups of 10 mice each (*Ipsen*, Protocol No. 97). The death time in minutes (T) was observed in case of each animal. Since it was obvious that T was not normally distributed, no rankit curves for T were drawn, but instead for $-\log T$, $100/T$, and $\log (415/T + 1)$. It will be seen that $\log T$ appears to be normally distributed in the case of the two highest doses, while this function becomes more and more asymmetrically distributed for smaller doses, *i. e.*, higher death times. The last dose killed only 5 of the mice; here we have a so-called truncated distribution, but this too is distinctly asymmetrical.

The function $100/T$ gives rankit diagrams which it is barely possible to fit to straight lines, best in the case of the lowest doses. The standard deviation is, however, to a high degree dependent on the magnitude of the observation, and the function is therefore unfit for comparison of means, the statistical theory for such comparison

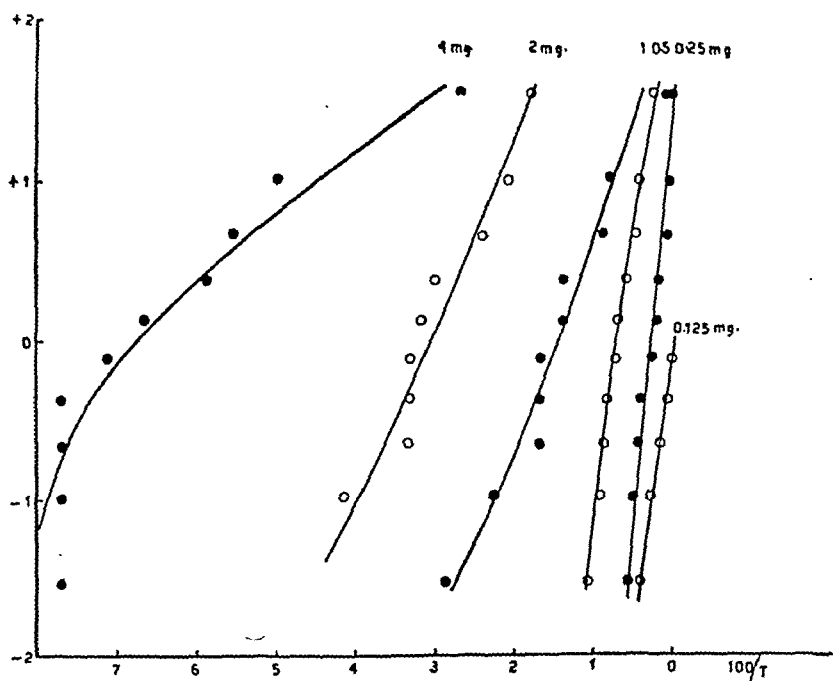
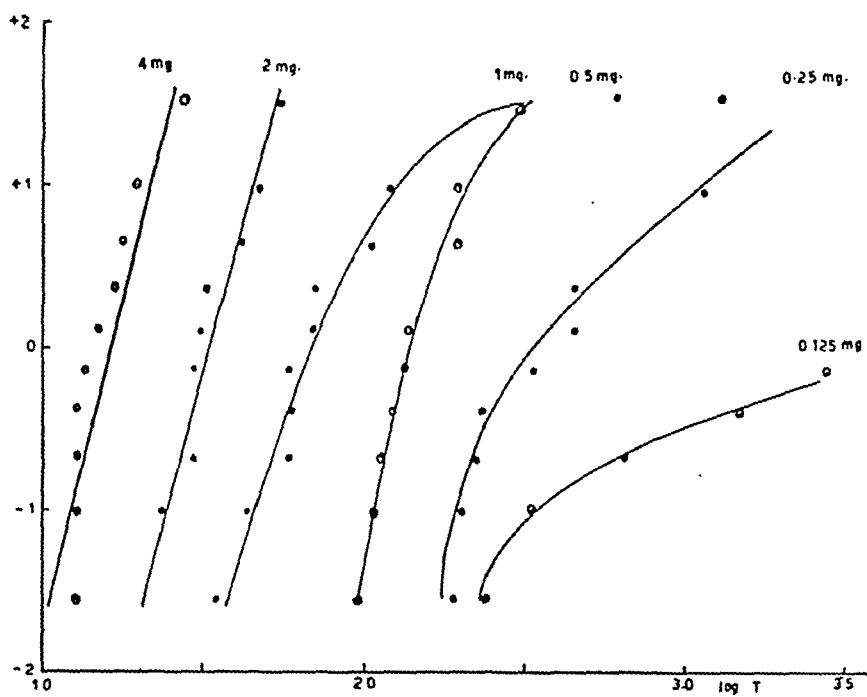


Fig. 4.

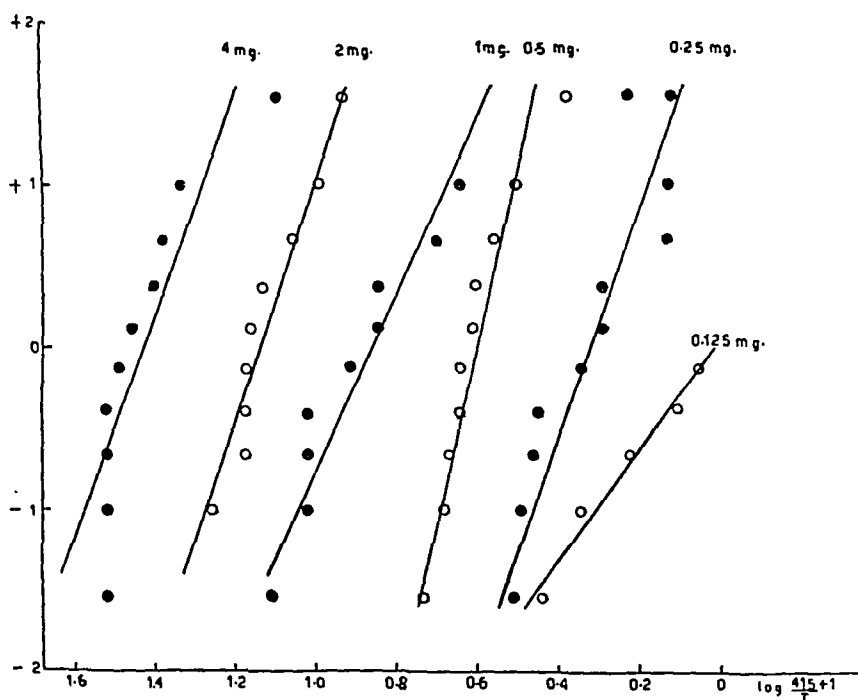


Fig. 4.

making it a condition that the standard deviation in the groups which are to be compared theoretically must be assumed to be identical.

The function $\log (415/T + 1)$, however, shows rankit curves that all appear to be rectilinear and moreover mutually parallel so that one may assume that this function is normally distributed with a standard deviation which is independent of the location of the distribution in the range of observations. Besides, the function is biologically justified, as it will appear from the paper from which the data are taken (Ipsen, pp. 122 and 132).

2. *Determination of mean and standard deviation.* If the points in a rankit diagram may be fitted to a straight line, and the distribution therefore may be assumed to be normal, then the straight line through these points will intersect the 0-line at a point the abscissa of which is the mean of the distribution.

The standard deviation is calculated as the reciprocal value of the coefficient of direction. Two points are located on the line and their coordinates (x_1, \bar{u}_1) and (x_2, \bar{u}_2) read off. The standard deviation is then $\frac{x_2 - x_1}{\bar{u}_2 - \bar{u}_1}$. The simplest method is to find the points $(x_0, 0)$ and $(x_1, 1)$, in which case the standard deviation is equal to $x_1 - x_0$.

If any of the observations are located above or below a threshold value dependent on the method of measurement, the distribution is called *truncated*. Death time assays often present examples of this

kind, the agent frequently being dosed so that only some of the individuals in the group are killed. A truncated distribution may very well be normal, but the assay is not so arranged that it takes in all of the distribution. In these cases the rankit method is extremely useful. One ranks the data for which a value within the limits of observation was obtained and plots the diagram for the members of the group falling within these limits. The table of rankits is then to be entered for n equal to the total number of observations, including those falling outside the limits of observation.

From a straight line through these points one may form a judgement of mean and standard deviation which, as in the complete distribution, are determined from the point of intersection with the 0-line and from the slope. The arithmetical calculation of the parameters is rather complicated, and can only be made on the basis of the graphical evaluation (see *Bliss, 1937*).

3. *Grouped observations.* Very frequently observations are made discontinuously so that the individual experiments are recorded as falling in groups between certain limits. In this case the rankit curves give a better idea of the mean than the arithmetical calculation, especially since this kind of observations as a rule lead to truncated distributions. One may distinguish between two different kinds of grouping which should be considered separately.

a) The metric scale is continuous, but for practical reasons the measurements are made discontinuously, resulting in a grouping of the observations. This is frequently true of serological assays, as for example in the case of the sera listed in table 5 where the contents of diphtheria antitoxin are given in groups with widely separated group limits.

Table 5.
20 sera assayed for diphtheria antitoxin (*Claus Jensen, l. c. fig. 6*).

Antitoxin Units pr. c. c.	< 0.0005	0.0005 to 0.01	0.01 to 0.1	> 0.1
Number	2	13	3	2
Adjoining rank number	2	3 15	16 18	19
Rankits of adjoining rank number	-1.41	-1.13 0.59	0.75 1.13	1.41

This distribution is bilaterally truncated inasmuch as the assay is not extended beyond 0.10 or below 0.0005 antitoxin units. Graphically this distribution is reproduced by plotting the 3 group limits 0.0005, 0.01 and 0.1 as abscissae, and at each of these values plotting

rankit of the two rank numbers which are just falling short of and exceeding the limit in question. One may also simply plot the mean of these two rankits, but the result is in practice the same. Through the three point-pairs or points the best fitting straight line is then drawn, the intersection of this line with the 0-line giving the median and the slope of the line giving the standard deviation.

b) Another kind of grouping a data occurs when the intensity of the observation is not measured quantitatively, but it is possible to establish so distinct qualitative differences between the observations that one may define degrees which may be designated numerically by whole numbers 0, 1, 2, By means of these intensity designations it is then possible to calculate a mean for the whole experimental series, which, as a rule, is a broken number. As soon as there occurs a large percentage of the observations with the smallest or the highest degree of reaction, the distribution must be considered truncated, and it is therefore expedient to judge whether the degree of intensity is normally distributed. If it is not, one must lump the two intensities together, or divide one of them into two, until the rankit curves are straight lines. It is obvious that all experimental series should be included in this investigation. It is surprising to see how often the experienced biologist »instinctively« selects exactly such degrees that the experimental data expressed in these units are normally distributed upon the first examination.

Table 6.

Cytological degree	0	1	2	3	4	5	6
Number	—	3	4	9	6	2	—
Adjoining rank number		3 4	7 8	16 17	22 23		
Rankits of adjoining numbers		—1.24	—1.04 —0.6	—0.48 0.37	0.48 1.24	1.50	

As an example of this kind in Danish literature we may mention the investigation by *K. Rosenquist* on endemic goiter where the clinical symptoms of goiter were divided into 5 degrees from 0 to 4, and the degree of goiter in a population group could be designated by a mean value. These values proved to be normally distributed. We may also mention *Ib Andersen's* graduation of histological changes in the thyroid gland in guinea-pigs treated with thyrotropic hormone. In

this case it was possible to establish 7 cytological qualities. One group of guinea-pigs was, for example, distributed as in table 6.

If this distribution is to be recorded in a rankit curve the procedure is as in the example in table 5. Rankits are plotted against the group limits until the number just falling short of or exceeding the limit, but it must here be realized that the group limits are the degree plus 1/2. The graduation is originally discontinuous, but is treated statistically as if continuous, and hence, for example, the degree 1 will denote the observations falling between 0.5 and 1.5. For that reason rankits of rank numbers 3 and 4 will in the example be plotted against degree 1.5, rankits of 7 and 8 against 2.5, etc.

The circumstance that we use the *mean* of rankits of the two rank numbers adjoining the group limits makes it possible to use instead, with good approximation, probits of the cumulative percentages *without* Bliss' correction. Inasmuch as we are to use the percentage corresponding to the limits between the groups that contain more than 0 observations, the frequency 100 % will never occur. Since it is in the case of the first and last rank number that the difference between probits and rankits is noticeable, there will but rarely be any need of the correction mentioned on p. 11, as there most frequently will be more than 1 observation in the extreme group. When the total of observations is less than 20 it pays to use the rankit method.

In practice one may use probit of cumulative frequencies instead or rankits for $n > 25$, if the percentage is calculated as $\frac{n-r+1}{n} \cdot 100$ when the observations are grouped, and as $\frac{n-r+1/2}{n} \cdot 100$ when the observations are continuous.

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CHANGE OF CAPSULE IN THE PNEUMOCOCCI

By A. Langvad-Nielsen.

(Received for publication Jan. 18th 1944).

In 1928, in a work on pneumococcus types, Griffith reported the transformation in vivo of one specific pneumococcus type to another specific type by subcutaneous injection into white mice of R pneumococci of one type (0.1 — 0.25 cc. of culture) together with the sediment from the heated and killed S pneumococcus culture of another type (in a dose of up to 100 cc. of culture). Cultures from the subcutis, heart's blood and peritoneum of the injected mice showed growth of capsulated pneumococci belonging to the same type as the vaccine. In this way Griffith succeeded in transforming R 2 pneumococci to S 1 and S 3.

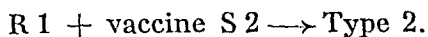
These findings of Griffith were confirmed and extended by Neufeld & Levinthal, Reimann, Dawson and others. As a rule the vaccine employed in those experiments was heated to 60°, from 15 min. up to 2 hours; when heated to more than 80° the vaccine was useless. The R pneumococci were obtained from the S pneumococci by cultivation either in homologous immune serum, in bile broth or in broth with addition of boiled pieces of organs (spleen, kidney or liver from rabbits or guinea-pigs).

Dawson & Sia were the first investigators for whom transformation of type in vitro turned out successful. The conditions for this transformation were minimal amounts of R culture, blood broth, addition of heated S pneumococci, small amounts of 10 % anti-R serum and incubation for a long period. With this technique Dawson & Sia succeeded in transforming R 2 to S 3 and S 1.

Baurhenn, Alloway and others confirmed the transformation of type in vitro. For Baurhenn the transformation turned out successful also with strains belonging to group X, as he was able to transform R 2 to S 8, R 6 to S 11 and S 29, and R 10 to S 29. Instead of vaccine, Alloway employed extracts of pneumococcal cells obtained either by repeated freezing and thawing of the bacteria or by dissolving the

pneumococci in solutions of bile salts and precipitating the activating factor with alcohol or acetone. In this way Alloway was able to transform R 1 to S 2 and S 3, R 2 to S 1 and S 3, and R 3 to S 1.

Schematically the transformation of type may be expressed in this way:



Naturally the main objection that may be raised to such a transformation of type will be that the vaccine was not sterile and that the growth of Type 2 pneumococci in the cultures from the mice originated from surviving pneumococci in the vaccine. Griffith, Reimann, Dawson and the other investigators carried out numerous tests concerning the sterility of the vaccine in vitro and in vivo but were never able to demonstrate the presence of living pneumococci in the vaccine. On the other hand, Haendel & Lange and, especially, Dunlop asserted that the vaccine was not sterile.

In order to prove the correctness of Griffith's report on transformation of type it was necessary therefore with absolute certainty to show that the vaccine employed was sterile or, preferable — what no investigator had done yet — to be able to prove that the body of the new-formed pneumococcus was the injected R strain. In order to prove the latter point, in my experiments I have used »marked« strains, *i. e.* strains with established constant properties connected with the body of the microorganism.

Technique.

The experiments in vivo were carried out in the same way as given by Griffith: subcutaneous injection of a small amount of R culture together with a large amount of heat-killed S vaccine; the injection was given to white mice, in the left groin. When the mice died subcultures were made from the subcutis, heart blood and peritoneum on blood agar and in serum broth; and after incubation of these cultures for 24 hours at 37° they were examined for the presence of capsulated pneumococci by employment of immune sera produced with the 2 S types from which the vaccine and R pneumococci originated.

The experiments in vitro were carried out by mixing vaccine, R culture, anti R-serum and blood broth in test-tubes and incubating the tubes at 37° for 15 days, subcultures being made every other day by transfer of 1 loopful of culture to blood agar and serum broth for examination for capsulated pneumococci; or the tubes were incubated for 24 hours, whereafter 1 loopful of culture was transferred to another test-tube containing the same amount of vaccine, anti-R serum and blood broth, and this procedure was continued for 5—10 consecutive days, and simultaneously with these subcultivations 1 loopful of culture was transferred to blood agar and serum broth, which on the following day were examined for the presence of pneumococci.

The R pneumococci were obtained by cultivation of the capsulated pneumococci in about 14 % homologous immune serum; the R cultures were about 8-hour cultures.

The S pneumococci employed for the S vaccine were high-virulent strains, recently isolated. The vaccine was produced in the same manner as given by Kaufmann, Bjørneboe & Vammen, and Mørch by cultivation in ox-heart broth. After centrifuging, the sediment was transferred to sterile ampullae, which were then sealed and completely immersed in a water-bath which automatically was kept at the temperature employed for the various experiments — as a rule, 20 min. at 60°. 1 cc. of the sediment corresponded to about 100 cc. of culture.

The resistance of the pneumococci to sulfapyridine (M & B) was determined after the method given by Schmith and the sensitivity of the microorganism was recorded in the manner suggested by him. Solutions of M & B in serum broth were made in 8 concentrations (1 : 5000, 1 : 10000, 1 : 20000 and so on, till 1 : 640000) and placed in a series of test-tubes which were numbered 1—8 with the increasing dilution of the M & B solution so that the first tube contained M & B in the strongest concentration, while tube 8 contained the highest dilution of M & B. When the sensitiveness of the pneumococcus to M & B was to be estimated, 0.05 cc. of a 24-hour culture in dilution 1 : 2000 was added to a series of M & B tubes which then were incubated at 37° for 48 hours. By numbering the tubes 1—8 the sensitiveness of the pneumococci could be expressed by the number of that test-tube which remained clear after 24 and 48 hours. Thus sensitivity 6 : 4 signified that tube 6 with the M & B concentration of 1 : 160000 was clear 24 hours after inoculation of the tube with the pneumococcus culture, and that tube 4 with the M & B concentration 1 : 40000 was clear 48 hours after the inoculation.

The fermentation power of the pneumococci was examined by transfer of 1 loopful of fresh pneumococcus culture to Hiss' medium (1 part of ox serum and 2 parts distilled water) with addition of the substances to be fermented in a concentration of 0.5 % and bromocresol purple for indicator and incubation at 37° for 14 days. If fermentation took place, the medium coagulated and became light yellow in color.

Sterility of the Vaccine.

In order to establish the sterility of the vaccine, I have carried out altogether 2917 sterility tests in vitro and 533 in vivo with pneumococcus vaccines of Types 1, 2, 6, 8, 14, 20, 22 and 35. The tests in vitro were performed by addition of various amounts of the vaccine (from 1 loopful to 3 $\frac{1}{3}$ cc.) to serum broth, ox-heart broth, horse serum broth, Martin broth, trypsin broth, blood broth, thin agar and 5 and

10 % blood agar plates, and incubation at 37° in ordinary atmospheric air, under anaerobic conditions or in CO₂ atmosphere.

The tests for sterility *in vivo* were carried out by injecting from 0.10 cc. to 1.0 cc. of the vaccine subcutaneously on white mice; the vaccine was injected either alone or together with other bacteria (colon bacilli — streptococci — staphylococci); in some experiments the mice were treated with an injection of mucin or alcohol prior to the injection of the vaccine. If living pneumococci were present in the vaccine the virulence might conceivably have been lowered to such an extent that they were not able to produce a fatal septicemia in the mouse but were killed themselves by the mouse. If, on the other hand, the resistance of the mouse was lowered at the same time by injection of R pneumococci, other bacteria or foreign substances, the resistance might perhaps be lowered so much that the injured and less virulent S pneumococci in the vaccine would now be able to multiply and kill the mouse.

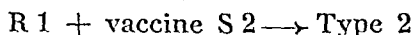
When the mice died or were killed autopsy was performed on them in the usual manner, cultures being taken from the subcutis, heart's blood and peritoneum on blood agar plate and in serum broth, which then were incubated and examined for growth of capsulated pneumococci.

All the sterility tests *in vitro* and *in vivo* were negative with exception of 2 experiments in which the results were uncertain. I shall not here enter into the details of these two tests but refer the reader to the account given of them in my previous work.

Transformation Experiments in vivo.

Even though the vaccines employed — apart from very rare exceptions — may be considered sterile, the transformation of type, as mentioned, would be established with greater certainty if the body of the new-formed pneumococcus might be proved to be the same as that of the R strain employed. In order to prove this I have made use of „marked“ pneumococcal strains, *i. e.*, strains in which established constant properties — as chemoresistance and fermentation power — are associated with the body of the pneumococcus.

From the schematic formula



it is evident that if the mouse experiments were carried out with employment of an R form with a chemoresistance or fermentation power differing from those of the S form, it would be practicable to decide from which of the two forms the body of the new-formed pneumococcus originated. If the new-formed pneumococcus possessed the same resistance or the same fermentation power as the type used for the vaccine, the pneumococci obtained in cultures from the mouse would come from surviving pneumococci in the heated vaccine. If,

on the other hand, the new-formed pneumococci showed the same resistance or the same fermentation power as the R strain, a transformation of type would have taken place.

The chemoresistance of the pneumococci is a constant property. Schmith and I have examined several pneumococcal strains a good many times at intervals of up to 2 years, after the pneumococci in the intervening period had been stored at room temperature as dried serum broth cultures or in icebox at about 4° in Truche with addition of gelatine, or after the strains have gone through numerous mouse passages and culture passages. In no instance, however, did a resistant strain become sensitive or vice versa.

Further, the R form of a pneumococcal strain proved to present the same resistance as the S form from which it was obtained.

Thus, after subcutaneous injection of a sensitive R 1 strain and a resistant vaccine S 2 into white mice, cultures from these animals yielded Type 2 pneumococci that were sensitive to M & B. This experiment indicated that a transformation from Type 1 to Type 2 had taken place; but the converse experiments with a resistant R 1 and a sensitive vaccine S 2 had to be carried out before the transformation of type could be said with certainty to have taken place. For it was conceivable that during the heating the S pneumococci might have become sensitive to M & B. But, as the mouse experiments with R 1 resistant and vaccine S 2 sensitive yielded resistant Type 2 pneumococci, a transformation of type must have taken place — or rather, attachment of the capsular substance of the vaccine to the R pneumococci. For it might hardly be imagined that the heating of the culture would sometimes alter its sensitivity to M & B from being sensitive to becoming resistant and at other times from being resistant to becoming sensitive, when the experimental conditions were the same.

Besides the transformation of Type 1 to Type 2, the following experiments turned out successful too: R 2 sensitive + vaccine S 1 resistant → Type 1 sensitive; R 2 resistant + vaccine S 1 sensitive → Type 1 resistant and R 1 sensitive + vaccine S 8 resistant → Type 8 sensitive.

Like the resistance of the pneumococci to M & B, also their fermentation power is a property connected with the body of the organism. In numerous experiments with 137 strains of 64 types, in which the strains were transferred to media containing various sugars and alcohols, some pneumococcus types were found to ferment certain of these substances while other types did not ferment them; and these experiments turned out in the same way repeatedly. By subcutaneous injection into mice of the R form of a pneumococcus types with a certain fermentation power together with an S vaccine of another pneumococcus type whose fermentation power differed from that of the R pneumococcus, it was now possible by determination of the

fermentation power of the new-formed pneumococcus obtained from the mouse to show whether a real transformation of type had taken place.

The experiments showed that R 1 always fermented inulin and never mannite, whereas S 2 never fermented inulin but nearly always mannite. The new-formed Type 2 pneumococci, resulting from R 1 and vaccine S 2 fermented always inulin but never mannite, *i. e.*, its fermentation properties were the same as those of R 1.

The experiments further showed that Type 6 never fermented inulin, whereas R 1 always fermented this sugar. In contrast hereto, the new-formed Type 6, obtained from R 1 and vaccine S 6, fermented always inulin, *i. e.*, in this respect it behaved like R 1, not like the ordinary Type 6.

The difference in fermentation power between Types 8 and 35 on one side and Type 1 on the other was too slight to serve as convincing evidence of the transformation of type, although Type 8 and Type 35 fermented mannite in respectively 50 % and 25 % of the tests, whereas Type 1 never fermented mannite. The new-formed pneumococci of Types 8 and 35 never fermented mannite.

Thus the experiments on the resistance and fermentation power of the pneumococci showed that a transformation of type or, rather, a change of capsule really took place, as the new-formed pneumococcus received its type-specific properties from the capsular substance of the vaccine employed and its species-specific properties from the R strain. The experiments further showed that the transformation from one type to another proceeded gradually, as some of the new-formed pneumococci on examination for capsular swelling with the homologous immune serum gave a distinct capsular swelling, whereas others showed merely a slight thickening of the capsule, and all sorts of variations were found between these two limits for capsular swelling. Nor was the capsular swelling always distributed equally over the individual pneumococcus as some of the pneumococci showed »bumps« in their capsular swelling. The appearance of the colonies of the new-formed pneumococci on blood agar plate was likewise subject to some variation, as the plates showed both large colonies which completely resembled the colonies of the S pneumococci employed for the vaccine and small colonies that were difficult to distinguish from the R colonies. Finally, the new-formed types were virulent to mice and presented all the other properties of the pneumococcus.

Experiments *in vivo* were carried out altogether on 1484 mice, but the attempt at transformation did not turn out successful in all the mice. When the experiments were carried out with »young« R pneumococci, *i. e.*, R forms obtained from S pneumococci after a few passages in homologous immune serum — in my experiments, less than 7 passages — the transformation turned out successful in almost every experiment and in some of the experiments in all the mice

employed, on an average in 32.5 %. When very young R pneumococci were employed, some R pneumococci would revert to the homologous type and, in the same mouse, others would be transformed to the heterologous types, while finally some would remain R pneumococci. When the R pneumococci were stable, *i. e.*, derived from S pneumococci after many passages in immune serum, the transformation experiments as a rule turned out negative — in my experiments transformation of type under such conditions took place on an average in 4.4 % of the mice.

Transformation Experiments in vitro.

These experiments — 787 altogether — were carried out in the same way as recorded by Dawson & Sia but the attempt at transformation of type did not turn out successful in any of them.

Summary.

By employment of marked pneumococcal strains, *i. e.*, pneumococci with constant properties connected with the body of the microorganism, it is shown that Griffith's transformation of type actually is a new formation, as the new type is made up of the body from one type and the capsule from the other type. Experiments on transformation of type turned out successful only *in vivo* — and most readily on employment of young R forms.

It is further demonstrated that this transformation proceeds gradually, as some of the new-formed pneumococci have only a little capsular substance distributed unequally over the body, whereas others completely resemble the S pneumococci employed for the vaccine, and that between these two limits pneumococci are encountered with all variations in the amount of capsular substance.

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FERMENTATION POWER OF PNEUMOCOCCI

By A. Langvad-Nielsen.

(Received for publication, Jan. 18th 1944).

The capacity of pneumococci for fermentation of certain »sugars« and alcohols under acid formation has been employed previously for identification of pneumococci; in particular, the behavior of the pneumococci towards inulin has been the subject of extensive investigation. Some authors claim that the capacity for fermentation of inulin is characteristic of the pneumococci and may be used as a criterion in the differential diagnosis for other bacteria (Hiss, Borden & Knapp, Avery, Chickering, Cole & Dochez, and Cooper, Mishulow & Blanc). In contrast hereto, other investigators assert that inulin fermentation is no absolute characteristic of pneumococci (Levy, Christiansen, Cotoni, Truche & Raphael).

In 1905 Hiss, Borden & Knapp examined the fermentation power of pneumococci for 11 sugars; and Christiansen (1913) investigated this property of pneumococcus Type 1 and a few pneumococci in the heterogeneous Group X with employment of 22 sugars, polyvalent alcohols and glucocides.

Comprehensive studies on the fermentation power of the pneumococci were first carried out by Viktorow, Semzowa & Sinjuschina (1934) and by Jürgens (1937). Viktorow and collaborators examined 32 strains of pneumococcus Type 1, 20 strains of Type 2, 11 strains of Type 3 and 25 strains of Type 4. Jürgens examined 122 pneumococcus strains of Types 1—3 and non-typed pneumococci of Group X.

These authors found that all the examined strains were capable of fermentation and acid formation in media containing d-glucose, d-mannose, d-galactose, d-fructose, lactose, saccharose, maltose, trehalose, raffinose, dextrin, glycogen and amygdalin, whereas they did not ferment l-arabinose, d-arabinose, xylose, l-rhamnose, erythrite, adonite, arabite, dulcitol, d-sorbitol, perseitol, inositol and arbutin. As to the fermentation of inulin, mannitol, melibiose, salicin and lactositol, the examined pneumococcal strains behaved differently.

Technique. — The present fermentation experiments were carried

out with employment of Hiss' medium, which was given by Hiss, Borden & Knapp and recommended by Levy, Christiansen, Viktorow, Semzowa & Sinjuschina and Jürgens.

Hiss' medium consists of one part ox serum and two parts distilled water. In the present experiments the substances to be fermented were added in an amount sufficient to make a concentration of $\frac{1}{2}$ %, and bromocresol purple was used for indicator. If the sugar in question was fermented, the acid production gave a fall in p_H , which originally was 7.8, and the bromocresol purple changed color, from blue to yellow, while the medium coagulated.

Instead of ox serum, horse serum was used in my experiments — it was also employed by Christiansen and Jürgens. The medium was placed in narrow test-tubes to a height of 2 cm.

The S pneumococcus strain which was to be examined was injected intraperitoneally into a white mouse in the evening; next morning the mouse was killed. Serum broth was inoculated with the heart's blood of the mouse, and the culture was incubated at 37°. With a Pasteur pipette, one drop of this serum broth culture was transferred to each of the tubes with the media, which then were incubated at 37° and inspected daily for the following 14 days. The cultures of R pneumococci were obtained by inoculation of serum broth directly from Truche cultures; otherwise, the fermentation media were inoculated and treated in the same way as the media inoculated with S pneumococci. Only a distinct change in color together with coagulation was reckoned as positive fermentation. From the tubes in which no fermentation appeared, numerous subcultures were made on blood agar plate, which each time showed growth of pneumococci. In the fermentation tubes the pneumococci were alive even two weeks after the inoculation.

The fermentation power of the pneumococci was investigated for the following 12 substances: arabinose, xylose, dulcitol, inositol, glucose, galactose, lactose, saccharose, inulin, mannitol, melibiose and salicin. For controls, Hiss' medium without any addition was inoculated with the respective strains.

Experimental Results. — All the pneumococcus strains in the collection of the State Serum Institute were examined in this way — a total of 137 strains of 64 types (the Danish strains and strains received from the Lederle Laboratories, New York). 42 strains were examined twice, a few strains three or four times.

None of the 137 strains was found to ferment l-arabinose, xylose, dulcitol and inositol, whereas glucose, galactose, lactose and saccharose were fermented by all the strains except Type 11, No. 34356, which did not ferment galactose. Galactose was fermented somewhat later than the other three sugars, which as a rule were fermented within 4 days after the inoculation.

The pneumococci differed in their behavior towards inulin, mannite, melibiose and salicin, but no definite characteristics in this respect could be demonstrated within the various groups. Thus 85 strains (62 %) and 39 of the types (60.9 %) fermented the polysaccharide inulin, while 25 types did not ferment inulin.

In contrast hereto, Jürgens found 97.3 % of his 112 strains examined were able to ferment inulin, but his strains belonged almost exclusively to Types 1, 2 and 3.

My experiments showed that not all pneumococci ferment inulin and, consequently, that inulin fermentation is not serviceable as a criterion in the differential diagnosis between pneumococci and other bacteria.

47 of the strains (34.3 %) or 19 of the type (29.7 %) fermented the alcohol mannite — and it was mostly the higher types that fermented mannite. 97 strains (70.8 %) or 53 types (82.8 %) fermented the trisaccharide melibiose; and 126 strains (92 %) of 61 types (95.3 %) fermented the glucoside salicin.

These experiments showed that serologically different types did not differ distinctly with regard to fermentation, and that serologically identical types did not always behave alike with regard to fermentation.

Besides this examination of the fermentation power of the capsulated pneumococci, also the R pneumococci of Types 1, 2, 3, 8 and 22 were examined many times for ability to ferment the same 12 sugars, and the experiments showed that the R pneumococci essentially fermented these sugars to the same extent as did the homologous S pneumococci.

The fermentation power of the pneumococci was found not always to keep constant for some of the sugars. On repeated examinations of the same strain at intervals of some weeks with the 12 above-mentioned sugars, I found that Type 1 fermented inulin only in 80 % of the tests, salicin in 40 % and melibiose in 6.7 %. Type 2, M57, fermented salicin in 40 % of the tests. Type 6, AL, fermented melibiose in 40 % of the tests, and salicin in 93.3 %. Type 6A, Walther Fiorelli, fermented mannite in 26.7 % of the tests, and melibiose in 53.3 %. Type 8, Henriques, fermented inulin in 60 % of the tests, melibiose and salicin in 66.7 %.

Summary.

64 pneumococcus types are examined for ability to ferment 12 different sugars and alcohols.

It is found that not all the types ferment inulin, and that inulin fermentation therefore is not serviceable as a criterion in the differential diagnosis between pneumococci and other bacteria.

It is further shown that serologically different types do not differ distinctly in their fermentation qualities, and that serologically identical types do not always behave alike fermentatively.

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STAINING OF THE DUODENAL GLANDS OF BRUNNER IN GROSS SPECIMENS OF THE DUODENUM IN MAN

A METHOD FOR STUDIES ON THE DISTRIBUTION OF THE GLANDS.

By *Erik Landboe-Christensen.*

(Received for publication October 4th 1943).

Studies on the quantitative distribution of the Brunner glands in a fairly large material would be difficult to carry through by means of the technique usually employed: embedding of fixed fragments of tissue, serial section on microtome and reconstruction. This method would be too troublesome and time-consuming.

For such studies the writer therefore adopted a technique analogous to the one employed by Maaløe (1908) and Hellman (1921) respectively for gross specimens of the appendix showing the distribution of the lymphatic follicles and of the total intestines for quantitative estimation of the lymphoid tissue.

Maaløe (1908) treated the appendix specimens with dilute acetic acid, which made the lymphoid nodules with their multitude of nuclei stand out conspicuously on the relatively clear background of the rest of the mucous membrane where nuclei are not so numerous.

Hellman (1921) elaborated this method further by combining the acetic acid clearing with hematoxylin staining of the follicles. Briefly, Hellman's method is as follows: The gut, which is opened along the attachment of the mesentery, is placed in running water for a few days. Then it is divided into sections, 15—20 cm. in length, and placed in 2—3 % acetic acid for 2—5 days, till it is quite transparent. Now the sections are placed in running water for 2—3 hours, then stained for 12—60 hours in Harris' hematoxylin diluted with distilled water (1:100). After this, differentiation is carried out with 2—3 % acetic acid, till the solitary follicles appear distinct on the almost unstained

background (12—24 hours). After washing, the serosa and muscularis are removed. The sections are then kept in Kaiserling III.

Hellman states that a numerical estimation of the follicles in the proximal part of the gut may be hampered a little by the presence here of the Brunner glands — but this is of minor importance as, in comparison with the follicles, the glands are stained but very faintly.

On employment of a stronger hematoxylin solution, however, together with a somewhat longer staining time and careful differentiation, the Brunner glands are found to become very conspicuous throughout the specimen. The glands occur as larger or smaller parenchymatous aggregates — »Brunner gland islands« (Landboe-Christensen, 1944) — which, being made up of epithelial cells, are more rich in nuclei than is the surrounding stroma, so that they react to treatment with acetic acid and hematoxylin after the same principle as the follicles, although not so strongly. The glands appear as more darkly staining granules on the background of the more faintly staining intestinal wall, especially the submucosa. The glands can be distinguished from the follicles by the deeper and more uniform staining of the latter, and also by their form: owing to their lobulation, the Brunner gland islands are very irregular in outline, while the follicles are regular, more rounded. Finally, their location is a distinguishing feature: the Brunner glands predominantly in the submucosa, the follicles in the lamina propria. This feature is seen most distinctly on examination of the specimen under binocular loupe at low magnification ($\times 4-20$).

The procedure adopted by the writer for staining of the Brunner glands in gross specimens is as follows:

1. On autopsy the duodenum is removed together with the adjacent part of the pars pylorica and of the jejunum. Without preceding fixation, the gut is laid open with scissors: the adjacent part of the stomach at the greater curvature, the duodenum in the midline of the anterior wall. The pancreas is removed; but, on account of the very tight attachment of the head of the pancreas to the descending part of the duodenum above, between and below the two duodenal papillae, not all the pancreatic tissue is removed here (beware of injury to the duodenal wall!). The common bile duct and the duct of Wirsung are not laid open.

2. Wash in running cold water till no more mucus is given off, generally 4—6 days.

3. Clear in 2—3 % acetic acid for 2 days.

4. Wash in running water for 6—10 hours.

5. Remove the remaining pancreatic tissue, the serous layer and as much of the muscularis as may be detached easily.

6. Stain with Harris' hematoxylin, 3—4 parts to 100 parts distilled water (or some other hematoxylin solution of the same strength), for up to 3—4 days. The staining takes place in large shallow dishes, in which the specimens can be spread out entirely. The specimens are turned over a few times.

7. Differentiate carefully in 2—3 % acetic acid for 6—24 hours.

8. Wash in running water for 6—10 hours.



Fig. 1.
Distribution of the Brunner gland islands in the proximal half of the duodenum from a girl, 15 years old (cause of death: lymphogranulomatosis). Specimen photographed under transillumination.

9. Remove carefully all remnants of the tunica muscularis.

10. Store in Kaiserling III with an addition of 0.5 % carbolic acid, preferably in refrigerator.

The stained gut is examined under transillumination: a box with a horizontal lid of opaque glass, the source of light being placed inside the box (*i. e.*, after the same principle as employed by Hellman for examination of the follicles). In most cases the Brunner glands may be examined best from the mucous surface of the specimen, so that they are seen through the mucosa. At the examination, the Kaiserling fluid is not removed, as it generally leaves the specimens sufficiently transparent. Particularly suitable specimens, or parts of them, may be photographed at the same time.

For examination of the glands in profile sections, portions of the gut are embedded in gelatin (after Gaskell — Gräff) in Petri dishes. After drying and denaturing of the gelatin, the block is cut in thin slices by hand, with a razor blade or knife.

This method permits a direct examination of the topographical aspects of the glands, including a precise determination of the proximal and distal delimitation of the Brunner gland region and the distribution of the glands. The outline, »form«, of the glandular islands may thus be observed readily, also their location with regard to the submucous blood-vessels and the plicae circulares (Fig. 1).

As to the amount of glandular tissue, it is not practicable directly to count the glandular islands or determine their volume, because they are extraordinarily irregular as to form, size and structure, besides being distributed very unequally, in some places very densely. But the method allows an estimation of the glandular density of the various parts of the duodenum, as it is possible after the proportion between the average size of the glandular islands and the average size of the free intervals between them to distinguish between different degrees of density. The varying density and the area of the various zones of corresponding density can be mapped out on the configuration of the specimen (Fig. 2).

Information about the size of the glandular islands is obtained by determination of their length and width, together with their depth (after embedding in gelatin). The surface area of the glandular region, especially its denser zones, together with the depth of the glandular islands, give an idea about the total parenchymal »mass« in the specimen.

The method offers a possibility of estimating the physiological variations in the amount of glandular tissue in the various specimens; it is also suitable for studies on hypertrophy and atrophy of the Brunner glands, and on the behavior of the glands in the case of scar formation (Landboe-Christensen, 1944).



Fig. 2.

Map of the distribution and density of the Brunner gland-islands in the specimen shown in Fig. 1. The two black spots indicate the location of the duodenal papillae; the arrows mark the pyloric ring and the beginning of the mesentery.

Density I signifies that the glandular islands lie so close-packed that the average size of the interglandular intervals does not exceed the average diameter of the islands.

Density II signifies that the intervals are wider than the glandular islands, but not over twice the diameter of the islands.

Density III means that the free intervals on an average are wider than twice the size of the islands, but not thrice.

Density IV indicates that the intervals are more than three times as wide as the islands, which are still distributed fairly equally.

Summary.

A method is given for staining of the duodenal glands of Brunner in unfixed gross specimens of the duodenum, after principles previously employed by Maaløe (1908) for demonstration of the lymphatic follicles in gross specimens of the appendix, and further elaborated by Hellman (1921) for quantitative estimation of the lymphoid tissue of the intestines.

The method consists in clearing of the unfixed and washed gross specimens of the duodenum in dilute acetic acid, overstaining with hematoxylin and conclusive differentiation with dilute acetic acid unto distinct appearance of the Brunner glands.

An account is given of the serviceability of the method for studies on the quantitative distribution of the glands, including the value of the method for the ascertainment of glandular hypertrophy and atrophy, besides the behavior of the Brunner glands in the case of scar formation.

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ABSTRACTS — ANALYSE — REFERATE

E. Haagen: Virukrankheiten des Menschen. Pp. 162. Steinkopff, Leipzig. 1941. 7.50 RM. A small unpretentious book that gives a survey of the more important virus diseases in a brief and clear form.

A. Jung: Die Funktionen der Vitamine des B-Komplexes im Organismus. Pp. 114. Hans Huber, Bern. 1941. 33.00 RM. A survey is given of the various vitamins of the B group and the diseases resulting from their deficiency, together with the relation of these vitamins to vitamins A, C, D and E and to insulin and adrenalin.

G. Fanconi: Die Störungen der Blutgerinnung beim Kinde mit besonderer Berücksichtigung des K-Vitamine und der Neugeborenenpathologie. Pp. 160. Thieme, Leipzig. 1941. 7.20 RM. *Fritz Koller*: Das Vitamin K und seine klinische Bedeutung. Pp. 150. Thieme, Leipzig 1941. 6.75 RM. The two Zürich clinicians Fanconi and Koller give a review of our present knowledge of vitamin K and its clinical employment, based in part on their own studies. Together, the two small books give a useful survey.

Emil Abderhalden: Abwehrfermente. 6^e ed. Pp. 150. Steinkopff, Leipzig. 1941. 6.00 RM. The author presents collectively his theory concerning Abwehrfermente and the clinical significance of these substances, which still appears somewhat disputed. The author emphasizes the importance of the fact that it is practicable now to demonstrate and isolate the »Abwehr« proteinases from the urine, and he gives the method for this.

Chemie und Krebs. Pp. 114. Verlag Chemie, Berlin. 1940. 2.10 RM.

The book contains 7 papers by various authors. Thus, *Butenandt* writes about biochemistry and the cancer problem; *v. Euler* deals with results obtained in enzyme chemistry and tasks of cancer research; *Lettre* treats on tissue culture as an adjuvant in chemical cancer research; and *Gross* on the significance of occupational toxicology to the development of cancer.

L. Haase: Über das Syndrom der Akrocephalosyndaktylie. Pp. 42. Fischer, Jena. 1942. 6.00 RM. A good survey is given of the present knowledge of acrocephalosyndactylism and other craniodysostoses, and it is pointed out that some forms of acrocephalosyndactylism, which are characterized in particular by symmetrical malformations, are hereditary, while other forms are due to exogenous factors, often presumable injury to the fetus in the first four weeks of pregnancy.

B. Falconer: Über die peptischen Läsionen. Pp. 66. Fischer, Jena. 1943. 6.00 RM. On the basis of autopsy material from St. Erik's Hospital, Stockholm, the author gives a statistic-etiological survey of peptic lesions. The investigation comprises 9300 autopsied cases and in 18 % peptic lesions were found. An account is given of the frequency of the disease in the two sexes, its various localizations and its etiology. The author points out that in the period of 1930—40 there has been an increase in the incidence of chronic ulcers and scar formation in the duodenum as well as in the stomach in males. The large material is worked up thoroughly, and the results are presented perspicuously. One only wonders why this publication on peptic ulcer in residents of Stockholm appears in the series on »Konstitutions- und Wehrpathologie«.

A. Rothmann: Spättd nach Kriegsverletzungen. Pp. 60. Fischer, Jena. 1942. 7.50 RM. The author analyzes 18 cases of late death in soldiers wounded in the war, and he divides them into 5 groups. In the first group, death was due to injury to the skull or brain; in the second, to functional disturbances of the urinary bladder; in the third, to vascular injuries; in the fourth, to sequelæ of gunshot wounds of bones; in the fifth, to injury to the trachea or main bronchi, in one case together with gas poisoning.

F. J. Lang: Pathologie der kronischen Gelenkleiden. Pp. 136. Steinkopff, Leipzig. 1943. 20.00 RM. In this book the well-known Innsbruck pathologist gives a useful little survey of the pathology of the various chronic joint lesions. After a brief general part, the special part deals with congenital joint affections, consequences of traumatic injury to the joints, deformities from overstrain, postural deformities, circulatory disturbances, and various forms of arthrosis and functional-mechanical arthropathy. Then mention is made of infectious and non-infectious, inflammatory lesions of the joints, neuropathic and endocrine joint affections, and finally, tumors and sequelæ from diseases in the periarticular tissue.

Gustave Roussy: Der Krebs. Pp. 290. Verlag Rascher, Zürich, 1943. This book is a German translation of »Le Cancer«, published in 1939 by the famous French investigator. It gives a comprehensive survey of the present state of cancer research and the combating of cancer.

After a brief historical introduction, mention is made of the various theories concerning the etiology of cancer; among others, a review is given of the significance of hereditary factors to the development of cancer, including the well-known mouse experiments in which it has been practicable through inbreeding to produce mouse strains in which cancer appears spontaneously in a very high percentage of the animals, and also the more uncertain studies reported on the inheritance of cancer in man — and the author emphasizes strongly the importance of additional new investigations for elucidation of this momentous question. Also the various, now largely abandoned, theories concerning microbes or parasites as causes of cancer are discussed. Finally, a review is given of the cancer-inducing capacity of various chemical substances.

Then the general biology of cancer is discussed, together with its anatomical and clinical features, besides our present knowledge concerning immunity and metastases in cases of cancer. A fairly thorough discussion is given of the possibilities in the treatment of cancer, and in conclusion a long chapter deals with the combating of cancer in various countries. Here mention is made also of the Scandinavian countries; but the author discloses that he is not so well-versed in geography as in cancer research, for

he believes that Greenland, Iceland and the Faroe Islands are inhabited by Lapps.

The book gives an excellent survey of the cancer problems, and even in the German translation its stamp of Gallic spirit and lucidity is conspicuous.

H. Albers: Kolloide, Elektrolyte und Hormone. Pp. 170. Thieme, Leipzig. 1943. Cloth, 12.60 RM. This work is an experimental study on the untoward effects of the female sex hormones, in particular, the influence on the serum colloids and the electrolytes of the blood, especially on the sodium-potassium-calcium balance. The technique of the various methods of examination is described. In conclusion, the author emphasizes that treatment with female sex hormones is no indifferent therapy, and that it interferes radically with many physiological functions and hence should be employed only when strictly indicated on the basis of an exact diagnosis; in particular, he advises against indiscriminate employment of sex hormones in the climacterium.

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VOL. XXI

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EINAR MUNKSGAARD · KØBENHAVN
MCMXLIV

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ACTA PATHOLOGICA ET MICROBIOLOGICA SCANDINAVICA

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In ACTA PATHOLOGICA ET MICROBIOLOGICA SCANDINAVICA only articles written by Scandinavian authors are published; they are issued in English, French or German, according to the author's desire.

Subscribers are requested to apply to *Ejnar Munksgaard*, Publisher, Copenhagen, Norregade 6. One volume (generally 4 numbers, ca. 6—700 pages) is published every year with numerous supplements. Each volume costs 35 Danish crowns.

Dans ACTA PATHOLOGICA ET MICROBIOLOGICA SCANDINAVICA ne sont publiés que des articles écrits par auteurs scandinaves; selon leur désir, ils seront publiés en français, anglais ou allemand.

Pour les abonnements on est prié de s'adresser au éditeur, *M. Ejnar Munksgaard*, Copenhague, Norregade 6. Prix par volume Cr. Dan. 35.—.

In ACTA PATHOLOGICA ET MICROBIOLOGICA SCANDINAVICA werden nur Artikel von skandinavischen Verfassern veröffentlicht; den Wünschen der Verfasser gemäss erscheinen sie in deutscher, englischer oder französischer Sprache. Zu beziehen von der Verlagsbuchhandlung *Ejnar Munksgaard*, Kopenhagen, Norregade 6. Preis pro Band 35 dänische Kronen.

I ACTA PATHOLOGICA ET MICROBIOLOGICA SCANDINAVICA optages som Regel ikke Afhandlingler større end 2 Ark (32 Sider). Manuskripter indsendes maskinskrivne, oversatte til Engelsk, Fransk eller Tysk, til en af de respektive Landes Redaktorer.

STATISTICAL POST-MORTEM STUDIES ON THE TENDENCY TO HEALING AND STENOSIS FORMATION IN GASTRIC AND DUODENAL ULCERS

By *Jens L. Hansen.*

(Received for publication Oct. 23rd 1942.)

Introduction.

In the course of studies on the changes in the appearance of peptic ulcer based chiefly on an autopsy material the writer arrived at some results concerning the tendency to healing of these ulcers and their tendency to stenosis formation that deviate on several points from the prevailing views concerning these features. In other respects, the results of this work confirm some hypotheses which have been advanced previously without being corroborated substantially because the materials of the respective authors were too small or investigated insufficiently. The autopsies here worked up by the writer constitute the most comprehensive pathologic-anatomical statistics reported so far on peptic ulcer, affording therefore a possibility of more far-reaching conclusions than have been warrantable so far.

The problems which have not yet been fully elucidated and which will be dealt with here are in particular:

1. The tendency to healing of the ulcers in the two sexes.
2. Tendency to healing of the ulcers in different localizations.
3. The juncture in the life of the ulcer patients when »hour-glass« stomach is most apt to be formed.
4. Sex distribution in the incidence of pyloric stenosis from juxta-pyloric and duodenal ulcers.
5. The juncture in the life of the ulcer patients when pyloric stenosis is most apt to be formed.
6. Frequency of stricture of the cardia from ulcer.
7. Causes of death in patients with hour-glass stomach and pyloric stenosis.
8. Frequency of cachexia as cause of death in ulcer patients.

Material.

The present material comprises a total of 21,200 autopsy records supplemented with the clinical diagnoses and other clinical data on adult patients (15 years old or more) who in the period of 1907—1936 died in the surgical and medical departments of the municipal hospitals of Copenhagen (the Kommune Hospital and the Bispebjerg Hospital). These hospital departments cover one-half of Copenhagen. Autopsy was performed on 80 % of all the patients who died in these departments.

Among the 21,200 autopsies, unquestionable, mostly chronic, ulcers of the stomach or duodenum or scars from such ulcers were observed in 1269 cases (6 %). Of acute ulcers, only large ones and such as were recorded as giving definite symptoms are included in this account — and only when they appeared not to be attributable to sepsis, uremia, circulatory disturbances, etc. The affections observed are divided into three groups according to their localization:

- 1) the corpus and fundus of the stomach;
- 2) the pyloric canal and pylorus; and
- 3) the duodenum.

The border between the fundus and pylorus is set at 4 cm. from the pylorus.

Tendency of the Ulcers to Healing.

An expression of the tendency of the ulcers to scar formation may be obtained by comparison of the number of scars with the number of ulcers. For, the greater the tendency to healing, the more scars in proportion to ulcers. This procedure has been employed in the autopsy statistics that have dealt with this question (Hauser, pp. 427 ff.). Whether it be serviceable in practice to give a correct idea of the actual proportion will depend on whether ulcers and scars on autopsy are observed with the same proportional frequency, that is, whether the two conditions appear equally distinct. This is hardly the case, however. At any rate, Hart (p. 291) claims that many pathologists will be apt to overlook a considerable number of smaller scars, especially in the duodenum (p. 293). If this be correct — as it seems to be (Hauser, p. 461) — the consequence will be that the healing tendencies found by means of ordinary autopsy materials are lower than presented by the actual conditions, especially as far as the duodenal ulcers are concerned.

An entirely different though probably less significant source of erroneous conclusions is afforded by the circumstance that the phenomenon which on autopsy appears as an ulcer is very likely in vivo in some cases to have been a scar, and vice versa.

In the enquiry into the tendency to healing, in the present work only lesions covered completely by epithelium have been reckoned as scars, whereas even the least defect in the epithelium is sufficient to make the scar formations be recorded as ulcers. As it was the aim of these studies only to investigate the natural spontaneous tendency to healing, all the cases have been ruled out that give

a past history of some operative treatment for the ulcer: gastro-enterostomy, resection, or suture of perforation. For the same reasons, cases of postoperative peptic ulcer of the jejunum have been omitted from this account.

In the present account, multiple ulcers and scars have been classified as follows: all the ulcers and all the scars are entered into the account; when the multiple affections had different locations, they are recorded under each of their locations; on the other hand, multiple ulcers or scars in the same location are calculated merely as one.

The sex distribution of the cases is given in Table 1. Here, as in the following tables, the tendency to healing is expressed by the cicatricial percentage (cic.%), that is, the percental frequency of scar formation in the total number of affections (ulcers + scars).

Table 1.
Sex Relation of the Tendency to Healing.

Character	Men	Women	Both sexes
Ulcers	455	302	757
Scars	183	352	535
Total	638	654	1292
Cic. %	29	54	41

From Table 1 it will be noticed that *the tendency to healing is considerably greater in the women than in the men*, the cicatricial percentages being respectively 54 and 29. This is also quite in keeping with the observation that the character of the lesion is more serious in men than in women (J. L. Hansen, pp. 40 and 102 ff.).

The influence of the localization of the lesion on the tendency to healing is dealt with in Table 2.

Table 2.
Relation of Location of the Ulcer to the Tendency to Healing.

Sex	Character	Corpus	Pylorus	Duodenum	Total
Men	Ulcers	171	135	149	455
	Scars	110	41	32	183
	Total	281	176	181	638
	Cic. %	39	23	18	29
Women	Ulcers	217	45	40	302
	Scars	318	21	13	352
	Total	535	66	53	654
	Cic. %	60	32	25	54

It will be noticed that the *corpus ulcers are most inclined to scar formation whereas the juxtapyloric and duodenal ulcers have a les-*

ser tendency to heal, and the cicatricial percentage for these ulcers being only about one-half of the cic.% for the corpus ulcers. This applies to both sexes (in the men the difference is four times greater than the standard deviation, in the women, five times greater*). *In all three locations there is a greater tendency to healing in women than in men.* This is in conflict with the results obtained from older autopsy materials — as shown in Table 3, which is a modified form of some collective statistics reported by Hauser (Table 13, p. 429; Table 16, p. 431 and Table 21, p. 461).

Table 3.
*Relation of the Location of the Ulcer to its Healing Tendency in
Some Older Statistics (Hauser).*

Sex	Character	Corpus	Pylorus	Duodenum
Men	Ulcers	370	27	193
	Scars	298	6	12
	Total	668	33	205
	Cic. %	45	48	6
Women	Ulcers	316	16	103
	Scars	794	10	3
	Total	1110	26	106
	Cic. %	72	38	3

The small number of ulcers and scars in the pylorus and pyloric canal is due to the fact that the figures for this location are taken from a single statistical account (Kossinsky) whereas the rest of the tabulation consists of data reported by 4—5 authors who made no distinction between juxtapyloric lesions of the stomach and proculapyloric. In Table 3 it will be noticed that the number of scars in the duodenum is strikingly low — which is probably due to the aforementioned source of error: that pathologists are apt to overlook scars in the duodenum. At any rate, this is suggested by Table 4.

In Table 4 the writer has recorded collectively the data on the frequency of ulcers and scars given in some more recent statistics, all based on very careful and thorough autopsies, namely the papers published by Hart (1919), Musa (1922), Gruber & Kratzeisen (1924) and Lehmann (1926).

Table 4 shows that in autopsy materials that are examined very thoroughly scars are demonstrable in the duodenum relatively just as often as in the stomach, so there is *no difference in the tendency to scar formation in the two organs*. As to the healing tendency in the pyloric canal and pylorus, no data have yet been reported in

*) Here, as elsewhere throughout this work, the standard deviation is calculated by employment of Yates' correction.

Table 4.

Relation of the Location of the Ulcer to its Healing Tendency as evidenced from Some Recent Statistical Data on Very Thorough Autopsies (collected by the Writer).

Sex	Character	Corpus (+ Pylorus)	Duodenum	Total
Men	Ulcers	91	75	166
	Scars	90	75	165
	Total	181	150	331
	Cic. %	50	50	50
Women	Ulcers	60	36	96
	Scars	194	73	267
	Total	254	109	363
	Cic. %	76	67	73

statistics based on very thorough autopsies, as the authors have not dealt with juxtapyloric and proculapyloric lesions of the stomach separately with a view to this particular feature.

The greater tendency to healing in women is confirmed in Table 4 both for gastric ulcers and for duodenal. *The healing tendency is about 50 % greater in women than in men*, that is, findings which are quite in harmony with the results obtained in the present material, as is particularly evident from Tables 2 and 5.

The influence of the age of the patients on the numerical proportions between ulcers and scars is presented schematically in Table 5.

Table 5.

Number of Ulcers and Scars in the Various Age-classes.

Age	Men				Women			
	Ulcers	Scars	Total	Cic. %	Ulcers	Scars	Total	Cic. %
15—19	4	1	5	13	3		3	27
20—29	22	3	25		8	4	12	
30—39	53	9	62	21	13	12	25	41
40—49	70	24	94		55	36	91	
50—59	112	49	161	28	59	59	118	55
60—69	119	43	162		70	100	170	
70—79	56	42	98	42	71	110	181	60
80—89	18	11	29		19	26	45	
90—99						5	5	
Unknown	1	1	2		4		4	
Total	455	183	638	29	302	352	654	54

It will be noticed that the number of scars in proportion to the number of ulcers increases steadily with advancing age. Here, of course, the cicatricial percentage is no direct expression of the healing tendency in the various age-classes. The relatively greater frequency of scars in the older age-classes is due to the circumstance that ulceration takes place less frequently in these patients while many of the ulcers of an earlier origin gradually heal, leaving the scars as permanent remnants, so that the scars accumulate with advancing age. Similar features are encountered also in other autopsy statistics (Hart, Gruber (a), Gruber & Kratzeisen, Kossinsky (p. 25) and Portis & Jaffé).

Bilocular Stomach.

Here the term »hour-glass stomach« is applied only to organs that have actually become deformed through scar formation, with a distinct narrowing of the lumen in connection with the presence of an ulcer or a scar. The present material includes 153 cases of this kind, in 9 men and 144 women. The far greater frequency of a bisaccate stomach in women is in harmony with all previous statements. In the statistical tabulation compiled by Hauser (Table 18, p. 452), based on 7 older autopsy materials, hour-glass stomach was observed in altogether 58 cases, including 7 men and 44 women, while the sex was not given in 7 cases. These 58 hour-glass stomachs were encountered among 1546 cases of peptic ulcer, making up 3.8 % of the total patient material. In the present material 153 hour-glass stomachs were observed among 1269 cases of peptic ulcer, that is, in 12 % of these patients.

The cause of this difference in the frequency of hour-glass stomach has to be looked for especially in the different sex and age distribution of the cases in the two materials (cf. Table 8), as the previous investigations comprise relatively more male patients and more younger persons than the present material. As hour-glass stomach occurs especially in women and elderly persons it naturally will be more frequent in this material than in the previous. This also explains why Grünfeld among the autopsies performed in the General Hospital of Copenhagen found an hour-glass stomach as often as in 30 out of 124 cases (24 %), as his statistics cover elderly persons alone. Strange to say, this point has not been taken into consideration before, and the high frequency observed by Grünfeld has been explained as due to the circumstance that he applied this term to too many stomachs, i. e., to stomachs which often were not sufficiently deformed for this designation (Hauser, p. 254).

This explanation cannot be refuted altogether, it is true, because the decision as to whether or not the term hour-glass stomach is to be applied in a given case is a matter of judgment insofar as

definite criteria have never been given for the definition of this phenomenon. But, in Grünfeld's material as well as the present, the large number of hour-glass stomachs is undoubtedly due primarily to the aforementioned differences between these materials and the material cited by Hauser, namely: differences in the age and sex distribution of the patients. An additional contributory cause of the

Table 6.

Frequency of Hour-glass Stomach in the Two Sexes in Relation to the Total Corpus Affections.

Character	Men	Women	Both sexes
Ulcers + scars	266	494	760
Bilocular stomach	9	144	153
Bilocular stomach %	3.4	29.2	19.9

great frequency of hour-glass stomach in the present material as compared to the others is found in the circumstance that our material comes from surgical and medical departments, where the symptom-giving cases of peptic ulcer cumulate, whereas the other materials were collected from all the autopsies in the respective pathological institutes.

The frequency of hour-glass stomach in the two sexes in proportion to the total number of ulcers and scars in the corpus of the stomach is shown in Table 6.

In the total material of corpus affections, then, an hour-glass stomach was seen in about one-fifth of the cases (19.9 %). *The tenden-*

Table 7.

Age Distribution of Cases with Hour-glass Stomach and of the Total Cases with Corpus Affections.

Age	Men			Women		
	Ulcers + scars	Bilocular stomach		Ulcers + scars	Bilocular stomach	
		No.	%		No.	%
15—19	2			3		
20—29	13			9	1	
30—39	19	1	2	16	5	31
40—49	44			68	21	
50—59	60	4	4	79	19	35
60—69	70	1		128	32	
70—79	44	2	5	147	51	35
80—89	14	1		38	13	
90—99				5	2	
unknown				1		
Total	266	9	3.4	494	144	29.2

cy to hour-glass stomach is several times greater in women than in men. This may be due in part to the greater healing tendency in women that has been pointed out in the preceding. But this greater healing tendency alone will hardly explain the great number of hour-glass stomachs in this sex, as the cicatricial frequency for corpus affections in women in the present material is only about 50 % higher than in men (Tables 2 and 6) — and this applies to other statistics too (Tables 3 and 4). The great frequency of hour-glass stomach in women is therefore likely to be due also to causes other than the greater healing frequency in this sex.

The age distribution for the cases with bilocular stomach is given in Table 7; for the sake of comparison the total corpus affections are recorded too.

From Table 7 it is evident that there is no particular difference in the incidence of hour-glass stomach in the younger and older age-classes. This shows that *hour-glass stomach practically always begins to form in relatively young patients (under 30 years) hardly ever later in life*. Otherwise we should find the incidence of hour-glass stomach increasing with advancing age. This is confirmed by surgical and radiological studies (Finsterer, Rieder). In the pathologic-anatomical literature it has been asserted, however, that hour-glass stomach was seen mostly in middle-aged and elderly persons, suggesting that the formation of this phenomenon would take place preferably later in life — and this appears to be evident also from figures published, as recorded in Table 8.

Table 8.

Frequency of Hour-glass Stomach in Relation to the Total Number of Autopsies in the Individual Age-classes in Collective Statistics (Hauser) and in the Writer's Material.

Age	Hauser's statistics			Age	Writer's material		
	No. of autopsies	Bilocular stomach			No. of autopsies	Bilocular stomach	
		No.	%			No.	%
—20	4060		} 1.0	15—19	530		} 0.5
21—30	1190	5		20—29	1380	1	
31—40	1447	6	} 2.7	30—39	2120	6	} 4.9
41—50	1509	2		40—49	3390	21	
51—60	1290	3	} 3.3	50—59	4610	23	} 6.2
61—70	1071	5		60—69	4450	33	
71—80	636	8	} 11.1	70—79	3610	53	} 14.2
81—90	165	2		80—89	1110	14	
?	703			90—99		2	
Total	11071	31	2.08	Total	21200	153	7.2

Table 8 shows the frequency of hour-glass stomach in relation to the total number of autopsies in the individual age-classes and in the collective statistics recorded by Hauser (Table 19, p. 453) made up of the figures reported by Cohn, Greiss and Kossinsky. For comparison, the present material is tabulated in a similar manner. The figures cover both sexes, but a greater majority of the patients with hour-glass stomach was made up of women (in the collective statistics, 25 out of 31; in the writer's material, 144 out of 153).

It will be noticed that in both of these statistical accounts the incidence of hour-glass stomach is increasing steadily with advancing age. Apparently, as mentioned, this is in conflict with the aforementioned findings: that the formation of hour-glass stomach takes place almost exclusively in the younger years of the ulcer patient. This conflict is only apparent, however — the apparent disproportion being due to the difference between the juncture for the hour-glass stomach formation and its demonstration on autopsy. Like any other chronic manifestation of peptic ulcer, hour-glass stomach is also more frequent on autopsy with advancing age of the patients examined. When we want to get an expression for the age at which the ulcer patients preferably have the formation of their hour-glass stomach, the only correct thing will naturally be to consider the frequency of this deformity in proportion to the number of patients with ulcers and scars, not in proportion to the total number of autopsies. For this reason, only Table 7 is suitable for information on this point, not Table 8. The earlier authors who employed tabulations of the same character as adopted in Table 8 were therefore bound to arrive at the erroneous result that hour-glass stomach is formed more frequently later in life than is actually the case. For the proper realization of the pathogenesis of hour-glass stomach it is important to keep in mind the result of these considerations: that hour-glass stomach practically always begins to form even while the patients are young. The fact ascertained here might very well give rise to considerations concerning the pathogenesis of stenosis; this question will not be entered into here, however, as it would require investigations that would fall outside the plan of the present work.

In the present studies no distinction has been made between the various degrees of the narrowing of the lumen — and this, I think, applies to all the previous investigations of this kind. Very likely, if such a distinction had been made, it would often show a more pronounced stenosis in the older of the patients than in the younger, but this does not alter the significance of the fact established here: that the stenosis formation commences early in the ulcer patients who have stenosis.

After this it may prove interesting to see whether the occurrence of hour-glass stomach has undergone any alteration through the period here examined, 1906—1936. Table 9 shows the frequency of hour-

glass stomach in proportion to the total number of corpus affections in the two sexes in each of the three decades of this period.

Table 9.
Frequency of Hour-glass Stomach in Proportion to the Total Corpus Affections in the Three Decades.

Period	Men			Women		
	Ulcers + scars	Bilocular stomach		Ulcers + scars	Bilocular stomach	
		No.	%		No.	%
1907—16	61	3	4.9	180	46	25.6
1917—26	97	5	5.2	174	52	29.9
1927—36	108	1	0.9	140	46	32.8
1907—36	226	9	3.4	494	144	29.2

For the men the figures are too small to allow of any definite estimation of possible changes. For the women the frequency of hour-glass stomach appears to have been practically unchanged throughout the decades here considered; and this means that the *tendency of corpus ulcers to hour-glass stomach formation has remained unchanged throughout this period*. Accordingly, the deformity has become relatively far more infrequent nowadays, as the corpus ulcers have decreased greatly in frequency (J. L. Hansen, p. 52 ff.). This is shown in Table 10, where the number of hour-glass stomachs are recorded in proportion to the number of autopsies among which they were encountered.

Table 10.
Frequency of the Hour-glass Stomach in the Three Decades in Relation to the Total Number of Autopsies.

Period	Men			Women		
	Total No. of autopsies	Bilocular stomach		Total No. of autopsies	Bilocular stomach	
		No.	%		No.	%
1907—16	2572	3	1.2	1961	46	23.4
1917—26	4255	5	1.2	3548	52	14.7
1927—36	4496	1	0.2	4368	46	10.5
1907—36	11323	9	0.8	9877	144	14.6

According to Table 10, the frequency of hour-glass stomach in women in these decades has fallen off from 23.4 ‰ to 10.5 ‰ for the total autopsies, that is, to less than one-half. So, evidently a not inconsiderable change in the occurrence of the disease has taken place within these few decades.

Stenosis of the Pylorus or Superior Part of the Duodenum.

Here the organic cicatricial narrowing of the pylorus and of the superior horizontal part of the duodenum will be dealt with under one — and in the following, for the sake of brevity, be designated as pyloric stenosis — as they constitute a clinical unity; besides, it is often impossible to distinguish between them anatomically. The last-mentioned point is due to the fact that most duodenal ulcers are located quite near the pylorus (according to Portis & Jaffé, two-thirds of the duodenal ulcers are located less than 1 cm. from the pylorus), so that not infrequently the exact normal-anatomical border between the stomach and the duodenum in the pathologically deformed organs cannot be established. In 6 of the 55 cases belonging to this group, however, there was a distinct interval of 2—7 cm. between the pylorus and the duodenal stenosis. In this account a narrowing of the lumen has been reckoned as stenosis when passage of the little finger was impracticable; in about one-half of the cases, however, the narrowing was far more pronounced; as a rule, the lumen then would allow merely the passage of a lead pencil or a knitting needle.

The material includes 55 cases of pyloric stenosis, 36 in men, 19 in women. In contrast to the hour-glass stomach, then, pyloric stenosis is more frequent in men. In proportion to the 1269 cases of peptic ulcer, the cases of pyloric stenosis make 4.3 %. In other materials the incidence varies from 2.2 % to 6.1 % (Hauser, p. 457).

The frequency of pyloric stenosis in proportion to the total juxta-pyloric and duodenal scars is shown in Table 11 for either sex.

Table 11.

Frequency of Pyloric Stenosis in Relation to the Total Ulcers and Scars in the Pyloric Canal, Pylorus and Duodenum.

Character	Men	Women	Total
Ulcers + scars	336	112	448
Pyloric stenosis	36	19	55
Pyloric stenosis %	10.7	17.0	12.3

Thus, one-eighth (12.3 %) of the total juxta-pyloric and duodenal lesions have been associated with a narrowing of the lumen. Also the tendency to pyloric stenosis proves to be greater in women, but the sex difference is not so pronounced here as in the case of the hour-glass stomach. Still, the difference is 1.8 times the standard deviation, and hence it may be assumed to be real — with a considerable probability (about 90 %). The literature has brought hardly any data that can supplement this result; and corresponding calculations, I think, are practicable only on the rather small figures reported by Kossinsky, which are cited in Table 12.

These figures show rather the converse: a greater tendency to

Table 12.

Frequency of Pyloric Stenosis in Relation to the Total Ulcers and Scars in the Pyloric Canal, Pylorus and Duodenum as reported in Kossinsky's Material.

Character	Men	Women	Total
Ulcers + scars	57	32	89
Pyloric stenosis	10	3	13
Pyloric stenosis %	17.6	9.4	14.6

stenosis in the men — but this difference is smaller than the standard deviation, and thus it is not at all statistically tenable. So, from the data available so far, the result arrived at in this work is at present to be considered correct, namely: that the tendency to pyloric stenosis, just as the tendency to hour-glass stomach, is greater in female patients than in male with juxtapyloric and duodenal ulcers.

The age distribution of patients with pyloric stenosis is shown in Table 13, together with the age distribution for the total cases of ulcers and scars in the pyloric canal and duodenum.

Table 13.

Age Distribution for Cases with Pyloric Stenosis and for the Total Cases of Juxtapyloric and Duodenal Ulcers and Scars.

Age	Men		Women		Both sexes		
	Ulcers + scars	Pyloric stenosis	Ulcers + scars	Pyloric stenosis	Ulcers + scars	Pyloric stenosis	
						No.	%
15—19	2				2		
20—29	13		5	1	18	1	
30—39	43	6	9		52	6	} 13
40—49	56	7	13	3	69	10	
50—59	92	8	23	5	115	13	} 12
60—69	71	7	30	6	101	13	
70—79	45	6	23	2	68	8	} 14
80—89	12	2	8	2	20	4	
90—99							
Unknown	2		1		3		
Total	336	36	112	19	448	55	12.3

As in the case of hour-glass stomach, the frequency of pyloric stenosis (last column) is seen to be about the same in the various age-classes over 30 years. It seems safe, therefore, here too to assert that the *stenosis begins to form almost exclusively already at a relatively young age of the ulcer patient.*

We shall then see whether the frequency of pyloric stenosis has undergone any change in the decades here investigated. Table 14 shows the frequency of pyloric stenosis in proportion to the total affections

located in the pyloric canal, pylorus and duodenum in the two sexes in the three decades separately.

Table 14.

Frequency of Pyloric Stenosis in the Three Decades in Proportion to the Total Affections in the Pyloric Canal, Pylorus and Duodenum.

Period	Men			Women		
	Ulcers + scars	Polyric stenosis		Ulcers + scars	Pyloric stenosis	
		No.	%		No.	%
1907—16	50	9	18	23	5	22
1917—26	114	12	11	38	6	16
1927—36	172	15	9	51	8	16
1907—36	336	36	11	112	19	17

The changes shown in Table 14 are too small to allow of any quite definite conclusion, but the tendency to pyloric stenosis appears to have decreased a little in the period investigated. Considering the two sexes under one, the frequency in the first decade is 19.2 %, in the last decade, 10.2 % — out of 73 and 223 cases respectively (the difference is twice the standard deviation). The explanation of the decreased relative frequency is possibly to be found in particular in the circumstance that in the later years resection of the stomach has been performed in a considerable number of cases and that this has lowered the incidence of pyloric stenosis. Hence, there is no definite evidence to suggest that the tendency to pyloric stenosis might actually have decreased. The absolute number of cases of pyloric stenosis is increasing because the juxtapyloric and duodenal ulcers have become more frequent (J. L. Hansen, p. 52 ff.).

Stricture of the Cardia from Simple Ulcer.

Stricture of the cardia brought about by a peptic ulcer is a very rare phenomenon. In pathologic-anatomical ulcer statistics so far only one case appears to have been described. At any rate, Harsløf (1907) was able in his thorough and comprehensive review of the literature (p. 56) to find only one case of this kind published (by Starcke); otherwise he found this condition mentioned merely as a theoretical possibility. And, in his dissertation on the pathological anatomy of peptic ulcer (covering no less than 472 pages), with references to about 1600 works, Hauser (1926) says (*l. c.* p. 460): »...in den verschiedenen pathologisch-anatomischen Statistiken finden sich bei den für die Kardia verzeichneten Geschwüren und Narben nirgends Angaben, dass diese mit einer Stenose verbunden waren«. The number of autopsies reported in statistical accounts on gastric and duodenal peptic ulcers up to 1926 may be estimated roughly to make at least

200,000 with about 10,000 cases of the disease. This number has probably included about 500 ulcers localized to the cardia, which make up about 5 % of the total number of ulcers (Hauser, p. 372—373). So when stenosis has practically never been ascertained among so many cases, it is safe to say that the tendency to stenosis of the cardia is extremely slight.

Still, a few casuistic clinical reports have been made on cicatricial stricture of the cardia after simple peptic ulcer (*e. g.*, L. Müller, p. 44; Schirmer, p. 18 and Hauser, p. 522).

In the present material, strange to say, no less than three patients died from stricture of the cardia, by peptic ulcer, with clinical features reminding of cancer. Microscopic examination of the lesion was made only in one of these cases, but from the description in the autopsy protocol the lesion must be considered to have involved a simple peptic ulcer in the remaining two cases too. Two of the patients were treated with gastrostomy, the first of them had also been submitted to gastroenterostomy a few years before; he died shortly after the operation, of intercurrent pneumonia; the other patient died of hemorrhage from the ulcer shortly after the gastrostomy. The non-operated patient died of emaciation. The age of the patients at exitus was respectively 48, 51 and 88 years; they were all men.

Causes of Death in Hour-glass Stomach and Pyloric Stenosis.

Among the 153 patients with hour-glass stomach, 39 (25.5 %) died of peptic ulcer. For comparison it may be mentioned that of the total 786 patients with corpus affections, 270 (34.4 %) died of peptic ulcer. Apparently, then, ulcer and scar with hour-glass formation is a little less dangerous than ulcer and scar without this phenomenon. The explanation of this is to be found in the fact that hour-glass stomach is encountered mostly in women and elderly persons, in whom fatal instances of peptic ulcer, including scars, are relatively less frequent.

Of the 55 patients with pyloric stenosis, 43 (78.2 %) died of peptic ulcer, whereas among the 483 patients with affections in the pyloric canal, pylorus and duodenum, 277 (57.4 %) died of peptic ulcer.

All told, then, among the 208 patients who presented these forms of stenosis, 82 (39.4 %) died of peptic ulcer. The remaining 126 patients died of causes without any demonstrable connection with a peptic ulcer and without any clinical manifestation of this lesion during the terminal period of illness — apart from the three cases in whom the ulcer was recognized, and in whom it had no influence on the fatal outcome — as it had not given rise to any acute complication, hemorrhage or quantitative nutritional disturbances.

In Table 15, these 82 cases are recorded according to the causes of death.

Table 15.

Causes of Death in Cases of Hour-glass Stomach and Pyloric Stenosis.
(For correction, see the text below.)

Cause of death	Hour-glass stomach		Pyloric stenosis		Total		
	Men	Women	Men	Women	Men	Women	Both sexes
Hemorrhage	2	19	5	2	7	21	28
Cachexia		5	7	8	7	13	20
Perforation	2	5	6	2	8	7	15
Operation		3	7	4	7	7	14
Other causes	1	2	2		3	2	5
Total	5	34	27	16	32	50	82

»Operation« as the cause of death means that the respective patients died after an operation for peptic ulcer without any acute complication (chiefly perforation). »Other causes« signify either intercurrent lesion (most often pneumonia) that developed under the treatment of the respective patient for peptic ulcer as the primary lesion, or infrequent ulcer complications (incarceration ileus in adhesions, from a previous gastroenterostomy, etc.).

The numerical proportion between the various causes of death are not to be considered valid in general. For the material does not consist of relatively equal quota of patients from surgical and medical departments, as the former represent a district with about 300,000 inhabitants, while the latter represent on an average about 450,000 inhabitants (about 300,000 in 1907—16, about 500,000 in 1917—26 and 1927—36 (J. L. Hansen, p. 18)). So the figures for death due to perforation and operation are to be increased by about 50 % if they are to be compared with the figure for death due to hemorrhage. Evidently, then, these three causes of death together with cachexia are seen about equally often in hour-glass stomach and pyloric stenosis, taken under one. In the cases of hour-glass stomach, however, we meet with a distinct preponderance of deaths due to hemorrhage, corresponding to the fact that the corpus ulcer is most apt to take a fatal course through hemorrhage (J. L. Hansen, p. 67). In pyloric stenosis, on the other hand, death due to perforation and operation is most frequent, partly because the juxtapyloric ulcers have a greater tendency to perforation than have the corpus ulcers (J. L. Hansen, pp. 71—72), partly because their stenosis in this narrow part of the stomach more often requires operative measures on account of obstruction to the passage of the stomach contents. This anatomical explanation covers also the numerical preponderance of death from cachexia in the group of pyloric stenosis.

Frequency of Cachexia as the Cause of Death in Ulcer Patients.

Among the 1269 patients with gastric or duodenal ulcer or scar from such ulcers which make up the present material, the ulcer lesion was the cause of death in 538 (42.5 %). This case mortality is far higher than the death rate reported from most previous materials (cf. J. L. Hansen, pp. 130—131) but this is due to the fact that the present material comprises only patients from surgical and medical departments, where the fatal cases cumulate. Among these 538 fatal cases, 39 patients (or 7.3 %) died with features of cachexia — with emaciation and chronic anemia — but without manifest hemorrhage, and not in connection with operative measures. In 24 of these cases the clinical diagnosis was cancer of the stomach, abdominal cancer, etc.; in 7 cases cancer seemed the most probable diagnosis, although it was not considered quite sure. In more than one-half of these cases (Table 15) the autopsy revealed a marked stenosis, most often at the pylorus. In the remaining cases of this category the autopsy showed no stenosis — but most often hypertrophy and dilatation of the stomach — indicating that functional conditions must here have played the decisive role in the difficulties of passage.

Among the patients who died with features of cachexia, the youngest was 38 years, the oldest 88 years; and most of them were between 60 and 80 years old. The average age for the men was 70 years, for the women 66 years. Of these patients 19 were men, 20 women. It is hardly accidental that women make up the majority of the cachectic patients, although the men were in the majority in the other groups of ulcer deaths (335 men against 203 women). For, as demonstrated above, the tendency to stenosis is greater among the female ulcer patients.

It is worth noting that *the frequency of death from cachexia appears to be increasing*. Table 16 gives the total deaths from peptic ulcer in the three decades and and the incidence of cachectic deaths.

Table 16.

Frequency of Cachectic Death in Proportion to the Total Ulcer Deaths in the Three Decades.

Period	Total ulcer deaths	Cachectic deaths	
		No.	%
1907—16	123	4	3.3
1917—26	213	14	6.6
1927—36	202	21	10.4
1907—36	538	39	7.3

The rise of 3.3 % from 1907 to 1916 to 10.4 % in 1927—36 is statistically established insofar as the difference between the two percentages is a little over twice the standard deviation. The rise in the relative frequency of the cachectic deaths may hardly be due to

an actual increase in the frequency of this outcome, as this would require that the tendency of the ulcers to stenosis was increased in the later years — and Tables 9 and 14 have shown that this is not the case. The explanation is more likely to be found in the fact that nowadays such patients are hospitalized more often than previously.

The question then suggests itself whether there might not still be a good many ulcer patients with these clinical features who do not die in the hospitals but at home. It is reasonable to assume that these patients are hospitalized in the same degree as patients suffering from cancer of the stomach, as clinically they usually are taken to belong to the latter category. According to »The Causes of Death in the Kingdom of Denmark« (official statistical report), of the patients succumbing from cancer of the stomach in Copenhagen 1931—39, 59 % died in some hospital or other nursing institute, 41 % at home. It is reasonable, therefore, to think that only a little over one-half of the patients who die from peptic ulcer with cachexia are hospitalized. In contrast hereto, nearly all the other fatal cases of peptic ulcer die in hospital (91 % in Copenhagen in 1931—39, according to »The Causes of Death in the Kingdom of Denmark«). According to these figures, it is safe to estimate that not above 10 % but, rather, at any rate over 15 % of all fatal cases of peptic ulcer terminate with features of cachexia.

Summary.

This investigation comprises 21,200 autopsies on adults (from 15 years old) who died in the surgical and medical departments of the municipal hospitals of Copenhagen in the period of 1907—36. This material includes 1269 cases of gastric or duodenal ulcers or scars from such ulcers. On the basis of this, the writer arrives at the following results:

1. The tendency of the ulcers to healing is considerably greater in women than in men (Table 1). This applies to all localizations of the ulcer — proculapyloric and juxtapyloric gastric and duodenal (Tables 2, 3 and 4).

2. The tendency to healing is the same in the stomach as in the duodenum (Table 4).

3. Hour-glass stomach begins to form almost invariably while the patient is still young, under 30 years (Table 7).

4. Pyloric stenosis is relatively more frequent in women than in men with juxtapyloric and duodenal ulcers and scars (Table 11), in analogy to hour-glass stomach in patients with corpus affections (Table 6).

5. Pyloric stenosis begins to form almost invariably while the patient is still young, under 30 years (Table 13).

6. Stricture of the cardia from ulcer has been encountered three times in the total 211 cases of stenosis in this material, and it has been the cause of death in 3 of the 85 fatal cases of peptic ulcer in this material.

7. The ulcer lesion was the cause of death in 39 of the 153 patients with hour-glass stomach and in 43 of the 55 patients with pyloric stenosis. Hemorrhage, cachexia, perforation and operation are the most frequent causes of death; they are found about equally often in patients with stenosis (Table 15).

8. The number of deaths from peptic ulcer with cachexia appears to be increasing. In 1927—36 they made up over 10 % of all the fatal cases of ulcer in which autopsy was performed (Table 16). As probably a considerable part of such patients, nearly one-half, die at home — just like patients with cancer of the stomach, and for whom they are usually mistaken clinically — these deaths are more apt to constitute more than 15 % of all deaths from peptic ulcer.

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THE DIFFERENCE IN OSMOTIC RESISTANCE BETWEEN A_1 AND A_2 ERYTHROCYTES.

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(Received for publication Feb. 14th 1943.)

The first indication of a subdivision of the blood group A was made by *v. Dungern* and *Hirschfeld*²⁾ in 1911 on the basis of some absorption tests. It was *Landsteiner*⁷⁾ in U. S. A., *Thomsen*⁹⁾ and *Friedenreich*³⁾ in Denmark, and their collaborators, who in the course of their thorough investigations — carried out in 1928 to 1931 — verified the existence of two serologically different subgroups of A, after *Landsteiner*⁷⁾ designated A_1 and A_2 . The three-gene theory of inheritance of blood groups advanced by *Bernstein*¹⁾ was extended in 1932 by *Thomsen*, *Friedenreich* and *Worsaae*⁹⁾ to a four-gene theory with the genes A_1 , A_2 , B, and R, where A and B are dominants and R recessive, and A_1 dominant to A_2 . This theory of the inheritance of the subgroups of A was also confirmed through family investigations undertaken at the same time by *G. Waaler*¹⁰⁾ in this country, independently of the former investigators. This theory of the inheritance of subgroups is now commonly adopted as the correct one, and has been further extended to include additional subgroups of A, most recently by *Gammelgaard*⁴⁾ in his dissertation, distinguishing between six different subgroups of A.

Opinions are still divergent, however, with regard to the different serologic characters of A_1 and A_2 erythrocytes; whether the receptors are qualitatively different or only differ in quantity.

During the performance of more than 10,000 blood grouping tests in this laboratory the subgroups of A were also determined. In doing this, it was observed that among A_1 and A_2 erythrocytes kept as a clot in their own serum the erythrocytes of type A_1 were more quickly hemolysed than those of A_2 . The observations were made in the following way: The method employed included the determination of agglutinogens as well as agglutinins.⁵⁾ Among the samples on which the blood groupings had been completed, every day one sample of

group A₁ and one of group B were selected to be used the following day for test corpuscles. As samples showing no hemolysis were preferably chosen, these time after time turned out to be of type A₂ on closer examination, whereas the A₁ erythrocytes often already had a layer of pink-coloured serum above their surface as a sign of the incipient hemolysis.

As this greater fragility of A₁ corpuscles was repeatedly observed, it was natural to assume a lesser osmotic resistance in A₁ corpuscles than in A₂. In a preliminary test a number of A₁ and A₂ samples were exposed to the action of a series of sodium chloride solutions with arithmetically decreasing concentrations from 0.70 to 0.30 per cent and intervals of 0.04 per cent. It appeared that the initial hemolysis (the minimum resistance) was more easily read macroscopically with sufficient accuracy than the total hemolysis (the maximum resistance). To obtain a sufficient precision in the gradation of the results it was necessary to diminish the intervals between the saline solutions to 0.02 per cent. Even these preliminary tests appeared to show a lesser resistance in A₁ erythrocytes as compared to the A₂ erythrocytes.

Methods.

For the testing of the osmotic resistance of the erythrocytes, a series of solutions of *natrium chloratum Kahlbaum pro analysi* (Schering A. G.) were made. Primarily a stock solution of 0.70 per cent was prepared. Certain amounts of this solution were then diluted with distilled water in increasing amounts with an accuracy of 0.001 cc., making solutions of 0.68, 0.66, 0.64 and so forth with intervals of 0.02, to 0.30 per cent. The solutions thus prepared were kept in bottles with well-fitting glass stoppers to avoid evaporation.

The blood samples to be investigated were treated in the following way: the serum was pipetted off, and physiological saline (0.85 per cent) added. The sample was then vigorously shaken to liberate the corpuscles from the clot. After being filtered through glass cotton, the corpuscles were washed thrice in physiological saline, each time being separated from the washing fluid by centrifugalising for two minutes with a speed of 3000 revolutions per minute. From the close-packed erythrocytes we then prepared a 50 per cent suspension in physiological saline for immediate use.

For the actual tests 0.5 cc. of every solution of sodium chloride was pipetted into small test tubes, making a series of tubes with gradually differing concentrations. To each tube was then added one drop of the corpuscle suspension to be tested. The rack of tubes was thereafter left standing for half an hour at ordinary room temperature, and the test tubes were then centrifuged a short time to throw the erythrocytes to the bottom. Immediately afterwards, the result was read: *the concentration of sodium chloride in the test tube show-*

ing incipient hemolysis expressing the minimum osmotic resistance of the erythrocytes under examination.

Undoubtedly the greatest interest is attached to the examination of entirely fresh erythrocytes, in direct connection with the collection of the sample. As this was difficult in the present case, I have performed the experiments with blood samples collected 24 hours previously. They all came from professional donors in The Norwegian Red Cross Blood Transfusion Service and were sent to the laboratory for serologic syphilis controls. All samples were collected, kept and transported to the laboratory under identical conditions.

To this investigation the objection may be raised that the reading of the incipient hemolysis may be influenced by subjective anticipation, thus rendering the results unreliable. Everyone can convince himself, however, that the reading of the initial hemolysis in salt solutions with intervals of 0.02 per cent does not allow much doubt about the results, and a good check on the anticipation may easily be obtained, if the reader of the experimental results does not know the subgroups of the erythrocytes investigated.

Results.

A total of 80 samples of group A_1 and 43 of group A_2 were examined, and in addition a smaller number of O and B erythrocytes in order to obtain an impression of the osmotic resistance of these corpuscles compared to A_1 and A_2 . The distribution of the initial

Table 1.

The Distribution of Observations in the Different Saline Solutions.

Blood Group	0.46%	0.48%	0.50%	0.52%	0.54%	0.56%	0.58%	0.60%	0.62%	Number of Observations
A_1		4	12	17	30	10	5	1	1	80
A_2	2	9	18	9	4		1			43
O	4	6	5	7	4	2	2			30
B	6	5	5	1	1	1				19
Total: 172										

Table 2.

The Mean Values of the Minimum Osmotic Resistance.

Blood Group	The Mean Value
A_1	0.5335 ± 0.003037
A_2	0.5037 ± 0.003567
O	0.5100 ± 0.006233
B	0.4884 ± 0.006455

hemolysis in the different salt solutions within the different groups is shown in Table 1, and the mean values for the minimum osmotic resistance with their standard errors appear in Table 2.

As the osmotic difference between A_1 and A_2 corpuscles is not so great that it may be seen easily in Table 1, owing to the dispersion of the results, various statistical operations have been carried out to interpret the observations.

On the basis of the mean, standard deviation and number of observations of minimum resistance for A_1 and A_2 , hypothetical frequency distributions according to the normal distribution have been calculated. The observed and the calculated values are compared in Table 3 and Figs. 1 and 2.

Table 3.
The Frequency Distributions of Initial Hemolysis.
Observed and Calculated Values.

The Concentration of Sodium Chloride	A_1		A_2	
	Observed	Calculated	Observed	Calculated
0.44%	—	—	—	0.4
0.46%	—	0.7	2	2.8
0.48%	4	3.6	9	8.8
0.50%	12	11.1	18	14.1
0.52%	17	20.4	9	11.3
0.54%	30	22.4	4	4.6
0.56%	10	14.6	—	0.9
0.58%	5	5.7	1	0.1
0.60%	1	1.3	—	—
0.62%	1	0.2	—	—

Apparently the observed frequencies agree well with the calculated ones. To get a more distinct impression of the conformity to the normal distribution, we may apply the chi-square test on the fit of the observed frequencies; if m' be the observed and m the calculated frequencies in the observation series, then

$$\chi^2 = \sum \frac{(m' - m)^2}{m}$$

As a result of these computations we get the probability of a deviation by chance from the normal curve of about 0.60 for A_1 and 0.80 for A_2 . A deviation of that magnitude may be expected in 3 of 5

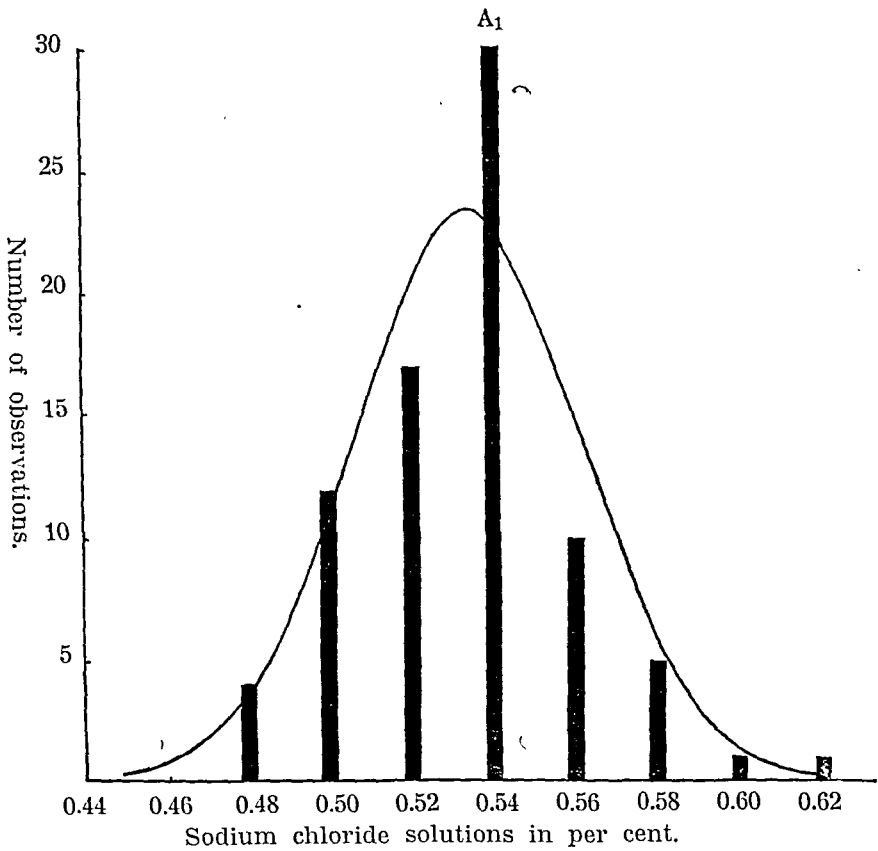


Fig. 1.

Observed frequencies of incipient hemolysis in A_1 blood corpuscles, compared with the normal curve.

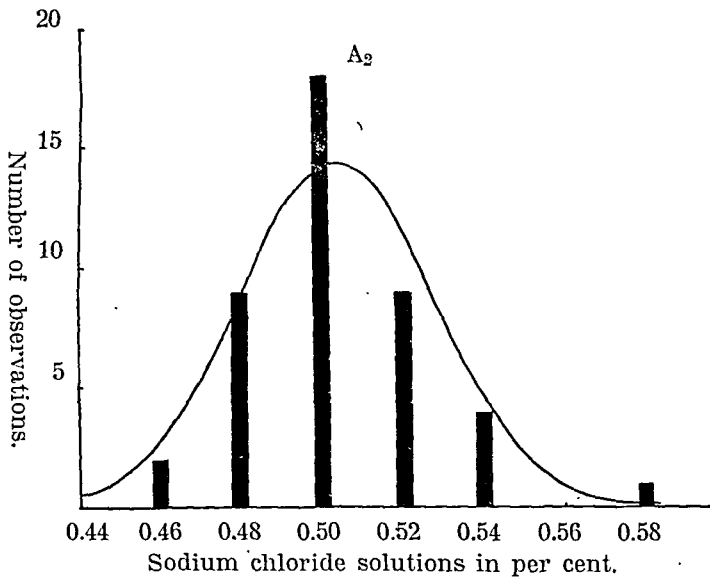


Fig. 2.

Observed frequencies of incipient hemolysis in A_2 blood corpuscles, compared with the normal curve.

cases for A_1 and in 4 of 5 cases for A_2 , in materials including the same numbers as the present one. Accordingly, the observed frequencies can be considered as having a normal distribution.

Assuming a normal distribution of the observed frequencies, it is justifiable to use the common statistical method with the calculation of the standard error of the differences between the mean values in the two observation series to test the significance of the differences in osmotic resistance between the A_1 and A_2 erythrocytes. As seen from Table 2, the standard error of the mean is for A_1 0.003037, and for A_2 0.003567, and accordingly the standard error of the difference is the square root of the sum of the squares of the two former values or 0.004683. As the difference between the means for A_1 and A_2 is 0.0298, it is 6.36 times greater than the standard error of the difference. A difference of 3 times the standard error is considered as significant; and the difference of 6.36 times the standard error may happen by chance alone only about 1 in 1,000,000,000, trials. We may therefore consider the observed difference in osmotic resistance between A_1 and A_2 erythrocytes to be statistically proved, presupposing a normal distribution of the observed frequencies.

It is also possible, however, to prove the difference between the osmotic resistance of A_1 and A_2 erythrocytes even if we do not accept the normal distribution in our material as established. This can be done with the chi-square test for the comparison of two observed samples, as this method does not imply a normal distribution of the two observations series. If in the expression below, n_1 and n_2 be the total of observations, and f_1 and f_2 the number of observations in the single classes in the two observation series, respectively, then:

$$\chi^2 = n_1 \times n_2 \times \sum \left\{ \frac{\left(\frac{f_1}{n_1} - \frac{f_2}{n_2} \right)^2}{\frac{f_1}{n_1} + \frac{f_2}{n_2}} \right\}$$

As a result we get $\chi^2 = 80 \times 43 \times 9908.1938 \times 10^{-6} = 34.08$, that is to say that the probability of a difference of this magnitude caused by chance alone is about 0.000,1. In other words, odds are about 10,000 to 1 that the difference observed in our material between the osmotic resistance of A_1 and A_2 corpuscles is a real difference, assuming the frequency distribution not to be in conformity with the normal curve.

Summing up, we may safely consider the observed difference in A_1 and A_2 corpuscles with regard to their fragility in hypotonic saline solutions to be statistically proved.

A smaller number of O and B bloods have also been examined in the same way (Table 1). As the numbers investigated are small and the dispersion about the means relatively great, especially with regard to the B erythrocytes, it is not justified to conclude too much from these materials. Looking back on table 2, however, we find that the

observed mean value of initial hemolysis in group O is situated between the means of A_1 and A_2 and closer to the latter. Applying the chi-square test for the difference of the two observed samples on A_1 and O, we get $\chi^2 = 21.72$. This is to say that the probability is less than 6 in 1,000 that so great a difference in the osmotic resistance is due to chance alone. Assuming the frequency distribution in the material of O bloods to be in conformity with the normal curve, and using the standard error of the difference between the means as a measure, we find that in less than 1 case out of 10,000 may so great a difference happen by the operation of chance.

Thus the difference between A_1 and O corpuscles with regard to

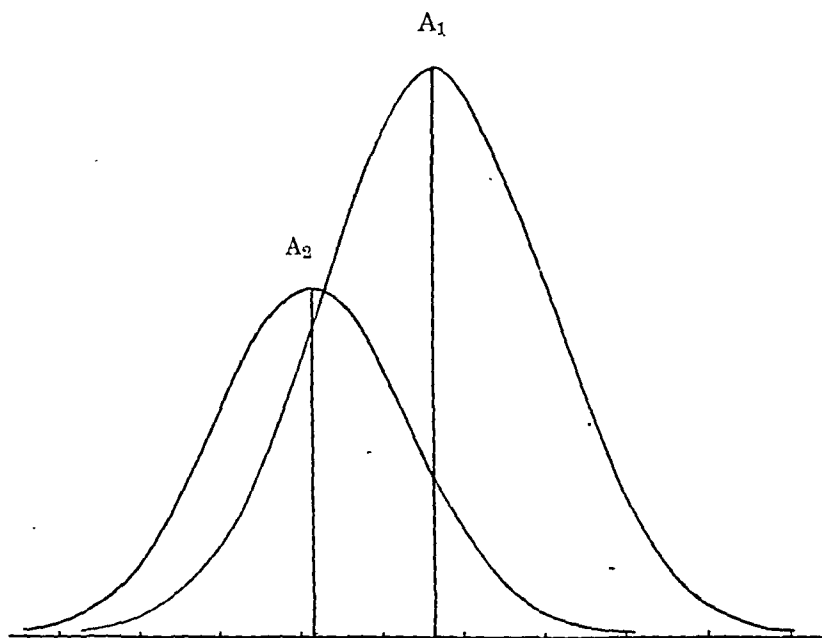


Fig. 3.

Normal dispersion of initial hemolysis in A_1 and A_2 blood corpuscles.
(See also Fig. 1 and 2.)

their fragility in hypotonic saline solutions is also statistically significant.

On the other hand, it is not possible in the present material to prove any difference in osmotic resistance either between O and A_2 , or between B and A_2 , or between B and O, whereas A_1 and B corpuscles show a distinct difference. The materials collected indicate, however, that the osmotic resistance of A_2 , O, and B are about of the same order. Minor differences may occur, but can only be investigated in a much larger material. Even in the present limited material it is striking that the O erythrocytes with regard to osmotic resistance are situated between A_1 and A_2 . This may be due to chance, but it is not very probable that the O erythrocytes actually will have a greater osmotic resistance than A_2 .

With the method employed in this investigation the erythrocytes showed an average initial hemolysis in salt concentrations of 0.50 to 0.55 per cent, whereas most authors⁸⁾ state the minimum resistance to be about 0.45 per cent. This lower resistance of the erythrocytes in the present investigation may possibly be explained by the fact that the blood samples had been collected the day before the examination, and the erythrocytes subjected to rough handling during the vigorous shaking of the clot and the repeated washing and centrifuging.

In Fig. 3 the calculated normal curves for the frequency distribution of initial hemolysis of A_1 and A_2 corpuscles based upon the present material are shown. It will be seen that the areas outlined by the two curves are partly coincident. Therefore it is not possible with the technique employed in these investigations to determine individual samples as A_1 and A_2 with the aid of the initial hemolysis in hypotonic solutions. This is also evident from the Table 1.

But if the erythrocytes had been examined in direct connection with the collection, without any rough handling as this then would be unnecessary, it is possible that the dispersion of the observations would be of a minor order. If the dispersion were of an order only allowing the curves to cross at the tails, the examination of osmotic resistance could be used as an additional characteristic in the determination of the subgroups of A. Investigations under these conditions have not yet been carried out, and it is therefore as well possible that the different behaviour of A_1 and A_2 corpuscles in hypotonic solutions is not developed in fresh samples, but develops only during storage, and due to other external influences.

We have not been able to detect any difference in resistance between women and men or between the subgroups M, N, and MN; but in our material there is a slight tendency toward increasing resistance with age.

The phenomenon described here is primarily observed in blood clots kept in their own serum and stored for a short time or some days. The difference demonstrated in the fragility of A_1 and A_2 corpuscles in this investigation is dependent upon certain other experimental conditions. More investigations under many different conditions are therefore necessary before the extent of the difference in the fragility of the erythrocytes in the two subgroups of A can be elucidated sufficiently.

Concerning the subgroups of A, two different opinions have been proposed. Most authors — including *Landsteiner*,⁷⁾ *Thomsen*⁹⁾ and *Friedenreich*,³⁾ basing their view upon very extensive and clever investigations — hold that the two receptors A_1 and A_2 are qualitatively different. On the other hand, the opinion has been advanced that the two receptors only represent different quantities of an identical antigen. As an exponent of the latter conception of this question, *Hirsz-*

*feld*⁶⁾ recently advocated an essentially different interpretation of the antigenic structure of blood groups. He states that only the group O was primarily present in the human race, and that the A and B properties originated by mutation in prehistoric times. In the mutations the O substance was not completely removed, but some O substance remained besides the A and B properties; in other words, some O substance is present in the genotypes AA, BB, and AB. Using anti-O serum produced by immunising goats with Shiga microbes, *Hirszfeld* stated he had demonstrated the presence of O substance in the B receptor and in different quantities in the receptors A₁ and A₂. *Hirszfeld* also claimed that the relative quantity of the O substance in the different groups could be demonstrated with great accuracy, as indicated in the following figure.

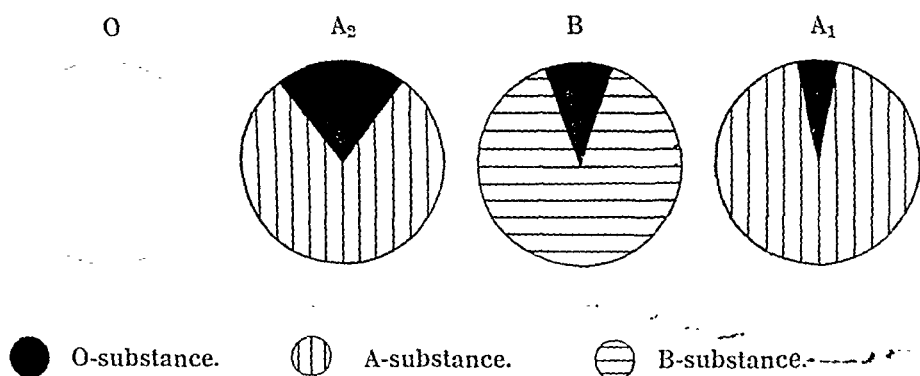


Fig. 4.

Content of O-substance in the different blood groups (from *Hirszfeld*).

As a conclusion *Hirszfeld* emphasises that the differences between the subgroups of A are only dependent upon the presence of greater or lesser amounts of O substance in the A factor.

The results of the present investigation can not easily be put in concordance with the *Hirszfeld* theory, whereas the phenomenon described here agrees well with the conception supported principally by *Landsteiner*, *Thomsen*, and *Friedenreich*: that the subgroups of A are qualitatively different.

Summary.

Different osmotic resistance in A₁ and A₂ blood corpuscles: On examining 80 samples of A₁ blood and 43 of A₂ under exactly the same conditions, it was found that the A₁ blood corpuscles were more easily hemolysed in hypotonical sodium chloride solutions than were the A₂ blood corpuscles (Tables 1 and 2). By a thorough statistical

sifting of the results it was found that this difference was so great that it could not be due to chance alone. It was further ascertained that A₂ blood corpuscles were not more easily hemolysed than O blood corpuscles. The observed phenomenon is hardly compatible with the view that the A properties are different only as to quantity, whereas it may easily be made to tally with the theory of a qualitative difference within the A properties.

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PERSISTENCE OF THE LEFT VENA CAVA SUPERIOR, WITH REPORT OF A CASE

By *Bodil Dohn.*

(Received for publication February 20th, 1943.)

Persistence of the left vena cava superior is probably the most frequent anomaly of the large veins. Thus Marshall (1850), Gruber (1864), Bauer (1896), Ancel & Villemain (1908) and McCotter (1916) have been able to collect altogether 120 cases. Subsequently several single cases have been reported.

The only case described by a Danish author dates back to 1871: »Aaben vena cava superior sinistra« (Patent vena cava sup. sin.) by Schmidt (Professor of Anatomy of the University of Copenhagen, 1866—1880). The heart, which came from a soldier who had never shown any sign of circulatory disturbances, was presented to the Anatomical Collection of the University; but, unfortunately, is no longer to be found.

Writer's Case.

The heart comes from a stillborn girl, at term, weighing 3300 g. and measuring 51 cm. in length.

The mother (Record No. 26317) was a healthy primipara, 26 years old. The gestation had been normal, and the pelvic measures were normal. Labor set in at term, with breech presentation. Delivery, by breech extraction, proceeded without difficulty. The fetal heart sounds had been good throughout labor; but when the child was born the heart action had ceased, and attempts at resuscitation turned out negative.

Autopsy was not performed, but as the presence of a congenital heart lesion was suspected, the heart was removed, and during this procedure it was noticed that the lungs were not distended. The large venous trunks were not inspected.

Description of the Heart.

The position of the heart and the pericardium present no abnormality. In comparison to a normal heart from a newborn with the same birth weight, the heart is small, relatively broad, and the apex is formed entirely by the right ventricle; but otherwise the heart is well-proportioned.

The weight and measures for the two hearts are as follows:

	Normal heart	Abnormal heart
Weight	16 g.	12 g.
Length	3.4 cm.	2.8 cm.
Width	3.5 "	3.6 "
Circumference	8.5 "	8.4 "
Diameter of:		
Vena cava inf.	5.4 mm.	5.4 mm.
» » sup. (right)	5.1 "	2.6 "
Inlet of coronary sinus	2.0 "	7.0 "
Vena cava sup. (left)		5.0 "
Pulmonary artery, ostium	5.4 "	5.4 "
Aorta	5.4 "	5.1 "
Wall of right ventricle	3.0 "	4.0 "
» » left »	4.0 "	3.5 "

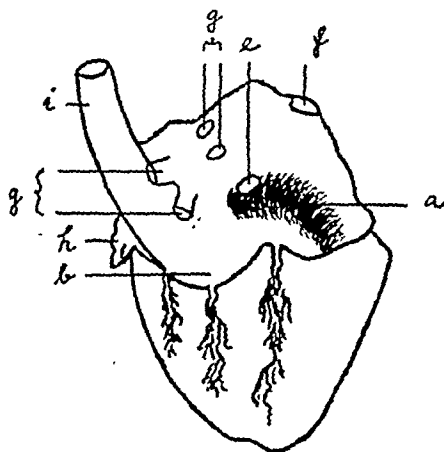


Fig. 1. Front view of the heart.

- a: Kidney-shaped depression on the posterior wall of the right auricle.
- b: Protrusion of the posterior wall of the right auricle.
- e: Inlet of vena cava inf.
- f: Inlet of right vena cava sup.
- g: Pulmonary veins.
- h: Left auricle.
- i: Left vena cava sup.

The posterior wall of the right auricle presents a large kidney-shaped depression (a), $13 \times 4 \times 4$ mm., with the concavity turning

downwards and to the left. In direct continuation of this to the left, the auricular wall is protruding (b); superiorly this protrusion covers the greater part of the auricular wall, inferiorly it is bulging over the auricular ventricular border. At its greatest width, this protrusion measures 13 mm. vertically. To the left, it continues between the auricles and ventricles till it turns up between the left auricular appendage (h) and the pulmonary veins (g), whereafter it continues vertically as a large vessel (i), left vena cava sup., the lumen of which measures 5.0 mm. in diameter.

The vena cava inf. (e) opens into the upper right part of the protrusion described. The inlet of the right vena cava sup. (f) is situated above, anteriorly and to the right of the inlet of the vena cava inf. On opening of the heart in the usual way, the right auricle proper is found to be normal in size and shape, whereas the more posterior part of the auricle — sinus venarum cavarum — with the inlets of the vena cava inf., vena cava sup. (right) and coronary sinus is considerably smaller and, especially, far more shallow than normally. This small, almost slit-formed, sinus venarum cavarum corresponds to the aforementioned depression seen on the posterior wall of the right auricle. The inlet of the vena cava inf. into the sinus venarum cavarum is bordered anteriorly by a well pronounced valvula venae cavae inf. (Eustachii), which continues normally in the terminal crest. Posteriorly to the left in the auricle, a large, funnel-shaped dilatation ($7 \times 7 \times 5$ mm.) is seen: the orifice of the coronary sinus. Inferiorly and anteriorly this dilatation extends in front of the valve of the inferior vena cava; to the left it reaches the auricular septum, just below the foramen ovale; and posteriorly it continues directly in the protruding sac described above. Valvula sinus coronarii (Thebesii) which normally is found anteriorly to the inlet of the coronary sinus, is here completely absent.

The foramen ovale is patent, 7×3 mm., sickle-shaped, covered only in part by an incomplete, pellucid and fenestrated valve.

The right ventricle is larger than the left, and its wall is thicker. The rest of the cavity of the heart, ostia, valves and other vessels show no abnormality.

The veins of the heart open normally into the coronary sinus, but they are protruding, dilated and somewhat tortuous before entering the coronary sinus.

Development of the Venae Cavae.

For the understanding of this malformation it will be appropriate briefly to mention some features in the development of the heart and large blood vessels.

At an early embryonic stage, the paired anterior and posterior cardinal veins (Fig. 2, a and b) conduct the blood back from

the head, neck and upper extremities — or from the primitive segments — and merge into the two short and thick ductus Cuvieri (c). The right and left ductus Cuvieri run ventrally and open into the right and left cornua (d_1) of the sinus venosus (d), the posterior venous section of the endothelial tube that forms the primitive heart.

Through the folding in the heart, the sinus venosus is located on the posterior wall of the auricle; and the inferior vena cava is brought to open into the cranial and right part of this sinus.

During the growth of the heart, the left cornu and the intermediate part of the sinus venosus are partially absorbed by the wall of the left auricle, leaving only the narrow channel, the later coronary sinus (Fig. 3, o), which opens into the right cornu (o_1). Subsequently

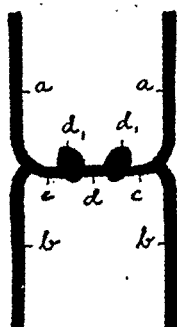


Fig. 2. Early anlage of the venous system in the fetus.

- aa: Anterior cardinal veins.
- bb: Posterior cardinal veins.
- c: Ductus Cuvieri.
- d: Sinus venosus.
- d_1, d_2 : Right and left cornua.

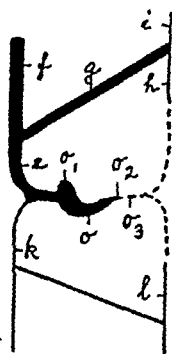


Fig. 3. The fully developed system in man.

- e: Right vena cava sup.
- f: Right vena anonyma.
- g: Left vena anonyma.
- h: Vena hemiazygos accessoria.
- i: Internal jugular vein.
- k: Vena azygos.
- l: Vena hemiazygos.
- o: Coronary sinus.
- o_1 : Right cornu.
- o_2 : Vena obliqua atrii sin.
- o_3 : Ligamentum venae cavae sup. sin.

the right cornu forms the posterior part of the right auricle, sinus venarum cavarum, into which the sinus venosus opens. Now a transverse anastomosis (g) is formed between the two anterior cardinal veins: the left vena anonyma. Normally it runs from the left cardinal vein downward to the right — something, as will be shown later, that is of importance to the further course of the normal development.

While the part of the left anterior cardinal vein that is situated between the transverse anastomosis and the heart, together with the left ductus Cuvieri (left vena cava sup.) is preserved as a functioning vessel in reptiles, birds and a few mammals, the greater part of it

undergoes obliteration in man and most mammals after the first fetal months. There then remains only the upper part (h) of the left anterior cardinal vein, which forms the vena hemiazygos accessoria, and the lower part. The latter may be demonstrated in a pericardial fold (ligamentum venae cavae sup. sin.) (o_3) as a fibrous cord, which on the heart continues in the vena obliqua atrii sinistri (Marshalli) (o_2), which opens into the coronary sinus.

Discussion.

The preservation of the left vena cava sup. as found in the heart here described is quite in agreement with the conditions in the human fetus in the middle or latter part of the 2' month. In the 6' month the left vena cava superior has already disappeared completely.

In the heart here described, as mentioned, the left vena cava superior is almost twice as large as the right.

As the cause of this malformation Ancel & Villemin mention four different possibilities, according to their findings in the individual cases, namely:

1. Absence of the anastomosis.
2. The anlage of the anastomosis is present but its development is poor, and its course is not from the left above to the right below, but transversal.
3. The anastomosis is well developed and is also running downwards to the right but more transversely than normally. The main bulk of the blood flows to the right through the anastomosis, but a part of it flows through the left vena cava sup.
4. The anastomosis runs from the right above to the left below, *i. e.*, the opposite of the normal course. As a result of this the right vena cava superior undergoes retrogression.

Unfortunately, as mentioned, at the removal of the heart no inspection was made of the large venous trunks, so that nothing is known about the course of the left vena anonyma, or whether perhaps it was completely absent.

The peculiar appearance of the posterior wall of the right auricle with the protrusion and depression described above is found also, though far less pronounced, in the normal heart of the newborn. When this relief of the heart here described is so conspicuous, it is presumably because the coronary sinus keeps conducting considerably more than one-half of the total amount of blood from the field of the vena cava superior to the right auricle so that its lumen — and hence the mentioned protrusion too (and the inlet in the auricle) — are bound to be of corresponding dimensions. Owing to such a dilatation of a living part of the auricle, the area to the right of this

will become markedly depressed, making the sinus venarum cavarum small and slit-like. However, this explanation is to be accepted only for what it is — speculation.

The anatomical structure of the heart at the different stages of the development is of significance to the direction taken by the blood stream in the heart. This was demonstrated by Schmidt in studies on fetuses of man, ox and sheep. He showed that at a certain developmental stage the inlet of the right vena cava sup. is situated below and to the right of the inlet of the inferior vena cava, and that the coronary sinus likewise opens into the auricle below and to the right of the inferior vena cava. In this way the blood stream from the superior venae cavae is directed straight to the right auriculo-ventricular ostium, without crossing the stream from the inferior vena cava that is directed against the foramen ovale. Also the shortness and width of the sinus venarum cavarum is of significance to the prevention of the blood streams from the veins being mixed during their passage through it.

The large funnel-shaped inlet of the coronary sinus in the present case is situated not only anteriorly, but also to the left of — and even a little posteriorly to — the inlet of the vena cava inferior. This may conceivably have brought about that the blood stream from this vessel was pooled with the blood stream from the vena cava inf., explaining thus why the foramen ovale here is so much larger than in the normal fetal heart. On the other hand, the stronger development of the right ventricle is rather suggestive of the opposite, namely: that the blood stream from the vena cava inf. is caught by the blood stream from the coronary sinus, which in this heart too is directed straight towards the auriculoventricular ostium.

As far as that goes, a persistent left vena cava sup. is no hindrance to the preservation of life; as the conditions for the outflow of the blood from the left vena cava sup. are just as good as those from the right. Indeed, several of the instances of this anomaly described in the literature were observed in adults. Hence this malformation is no obstacle either to the change in the circulation of the fetus at birth. As is well known, this change is introduced with the expansion of the lungs through the first inspiration — but this process has not taken place at all in the present case. So the death of the fetus can hardly have been due to the malformation of the heart and has to be put down as unknown.

Summary.

After a brief mention of the literature on persistent left vena cava sup., a description is given of such a case, observed in a stillborn girl at term.

For elucidation of the present case, mention is made of some developmental features, and an attempt is made through this to explain the anatomical features observed and their significance to the currents of the blood in the heart.

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A TRANSPLANTABLE CORNIFYING SQUAMOUS-CELL CARCINOMA IN MICE

By *J. Engelbreth-Holm.*

(Received for publication February 22nd, 1943.)

Among the spontaneous tumours transmitted from passage to passage in the laboratories all over the world, a carcinoma arising from cornifying squamous-cell epithelium has long been missing.

Thus, a brief report of such a tumour might deserve some interest. The tumour in question has been transplanted for years in our laboratories, primarily in the »Danish Anti Cancer League's Cancer Research Laboratory«, and now in the University Institute of Pathological Anatomy, Copenhagen. In the last place it is left to the disposal of anybody wanting to use it for further investigations.

The original tumour was found in a mouse (no. 5056) of the leukaemic strain Aka. This mouse was painted on the back with a suspension of 9:10-dimethyl-1:2-benzanthracene in benzene. No tumour-growth appeared in the painted area. After seven months, however, a subcutaneous round firm nodule was felt in the left cheek increasing quite evenly from day to day until it had reached the size of 1 by 2 by 2 cm. Then the mouse was killed. Tumour was located in subcutis, it was rather sharply outlined, homogeneously greyish-red on section, and of firm consistence. Its origin was obscure. There was no connexion between tumour and the skin, the latter being found quite intact. Most likely it had taken its origin from the mucous membrane of the buccal cavity or perhaps from a glandular excretory duct.

Histological examination of the tumour (fig. 1.) showed a typical squamous-cell carcinoma (of type I) with marked keratinization. In a stroma of connective tissue abundant irregular large isles of stratified epithelium with central cornifying masses displaying both a stratum germinativum and a stratum corneum were found. The epithelial cells were but slightly atypic and moderately polymorphic corresponding to the high stage of differentiation. Invasive growth

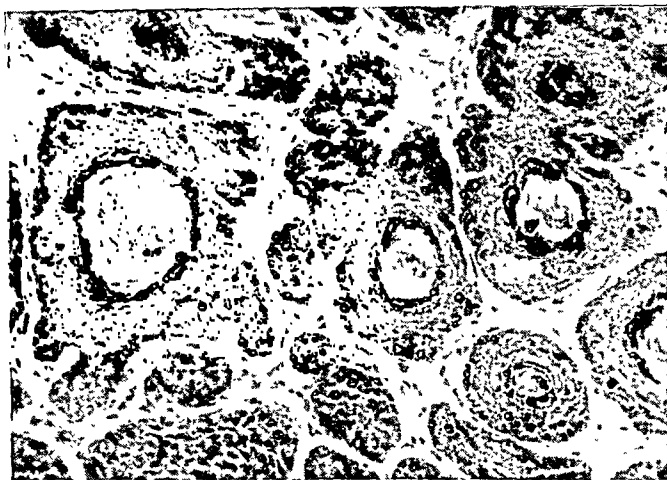


Fig. 1.

Cornifying squamous-cell carcinoma.
Spontaneous tumour from mouse of Aka-strain no. 5056.
× ca. 250.

was observed in the peripheric areas of the tumour, and on transplantation into seven mice of the same inbred strain, takes were found in all animals developing into enormous tumours that killed the mice within 4—5 weeks.

Since then, tumour has been transplanted through 62 passages, the taking percentage being 100 in a total of 600 mice of the Aka-strain, no successful transplantation having been obtained in untreated mice of other strains. The malignancy of the tumour is evidenced



Fig. 2.

Second passage. Mouse no. 10048.
Cornification is still pronounced.
× ca. 250.

— by the transplantation results, partly by the pronounced invasive growth (see fig. 4. Tumour has invaded the striated muscle layer), — and finally by the fact that metastases were found in some cases in the lymph nodes or in the lungs.

It is most interesting to investigate the alterations to which this tumour — like other transplanted tumours — has been subjected during transplantation. In the carcinoma in question the histological picture displayed a marked morphological transformation.

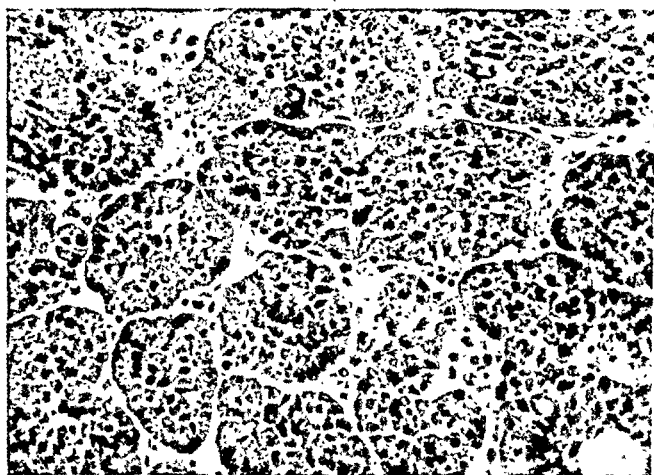


Fig. 3.
Fourth passage. Mouse Aka no. 6106.
Tumour almost purely basocellular.
× ca. 250.

It is a well-known fact that transplanted tumours will change during the passages, tending, as a rule, towards a greater virulence, viz: a more rapid killing of the animal and an increase of the percentage of takes. Various explanations of this fact have been discussed. Most probable seems the theory advocating that inhibition of the rate of differentiation, and perhaps of other cellular functions as well, may take place during the passage through various foreign organisms, thus, rendering the tumour more malignant; in other words: a tumour belonging originally to type I is gradually transformed into type II, III and IV.

This explanation seems the most adequate in the case in question. There is no reason to believe that the original tumour has consisted of a mixture of strongly virulent (= undifferentiated) and less virulent (= more differentiated) cells, ascribing thus, the changes during the passages to a »selection« of the most malignant cells. The fact that the most malignant cells are less vital than the less defective, more differentiated and, hence, less malignant cells, will render this supposition less probable.

Further, in the tumour in question we have no mixture of more or less malignant cells, but a fairly organoid mass of epithelial cells differentiating quite uniformly and side by side towards a flat keratinizing cell, forming, thus, the stratification that was so typical of the original tumour (see fig. 1).

Like other tumours, this squamous-cell carcinoma has changed its character during the passages developing into a less differentiated and accordingly more malignant type, under the picture of an ac-



Fig. 4.

28th passage. Mouse Aka no. 7823.
Basocellular tumour infiltrating striated muscle.
× ca. 250.

celerated growth, a greater tendency towards necroses and an abbreviation of the life time of the mouse.

Attention must particularly be focussed on the morphological changes of the tumour tissue. To point out histologically the altered differentiation of a solid carcinoma, or of a sarcoma, is difficult if not impossible, whereas the inhibited differentiation of the keratinizing squamous-cell carcinoma in question has found its distinct expression in the fact that the histological picture during the first passages was transformed from a keratinizing squamous-cell carcinoma (fig. 1) into a basal-cell carcinoma (fig. 3) displaying only traces of keratinization that was the predominant feature of the primary tumour.

It ought to be noticed that the basocellular picture does not resemble the so-called »Krompecher basal-cell carcinoma« in man, it corresponds entirely to the lowly differentiated squamous-cell carcinoma, as f. inst. seen in the oral cavity and in the oesophagus.

Moreover, the malignancy of the tumour is increasing, appearing as an abbreviation of the time of taking, and of the life time of the

mouse from three months to one month. It is, however, not till the later passages in which tumour had become quite basocellular that the mice into which the tumour was transplanted died within one month displaying large, partially necrosing, often cystic tumours growing invasively into the surrounding tissue.

Summary.

A transplantable carcinoma originating from squamous-cell epithelium in mice, is described.

Through numerous passages, a transformation of the tumour has taken place converting the tumour from a keratinizing into a basal-cell carcinoma, and rendering tumour coincidently more virulent, causing, thus, a decrease in the life time of the mice.

It is suggested that this transformation may be ascribed to an inhibition of the differentiation, of the tumour cells initiated by the transplantation of the cells into foreign organisms.

DIE MEGALOBLASTENFRAGE

(STUDIE ÜBER DIE REMISSION DER PERNIZIÖSEN ANÄMIE)

Von *Nils G. Nordenson*.

(Eingegangen bei der Redaktion am 26. Februar 1943).

Bei der Leberbehandlung der perniziösen Anämie entstehen im Blut und Knochenmark Veränderungen, welche ausserordentlich überraschend und auffallend sind. Die Remission keiner andern Blutkrankheit kann sich an Intensität auch nur annähernd mit derjenigen messen, die man bei perniziösen Anämie findet, und jeglicher Vergleich verblasst dagegen ganz und gar. Im grossen und ganzen hat man infolge der zahllosen Veröffentlichungen von dem Remissionsverlauf ein ziemlich klares und deutliches Bild. Nach wie vor sind jedoch dunkle Punkte vorhanden, die sehr interessant sind, und die wohl wert sind, von neuem zur Diskussion aufgenommen zu werden.

Das Wesentlichste in der Hämatologie der perniziösen Anämie ist die pathologische, megaloblastische Erythropoese, eine Blutbildung, die bei der Remission normalisiert wird und in die normale und normoblastische Erythropoese übergeht. Diese Normalisierung soll im folgenden eingehend besprochen werden. Das Studium wird sich auf Details sowohl im Knochenmark wie im peripherischen Blut stützen. Indessen möchte ich darauf aufmerksam machen, dass diese Details dem Fachhämatologen in den meisten Fällen wohlbekannt sind. Neue Details kommen demnach nicht in Betracht, sondern das eventuell Neue liegt in ihrer Deutung. Die Megaloblastenfrage bildet den Kernpunkt der Arbeit. Ausserdem dürfte es aber von allgemeinem medizinischen und klinischen Interesse sein, die Remission der perniziösen Anämie allseitig in einer Veröffentlichung zu beleuchten.

Es ist wohlbekannt, dass die Blutbildung bei der unbehandelten perniziösen Anämie megaloblastisch ist. Die Voraussetzung für die Entstehung einer perniziösen Anämie ist das Fehlen des endogenen

Faktors (»intrinsic factor«), dessen Vorhandensein demnach für eine normale Erythropoese notwendig ist. Aber für die Remission einer perniziösen Anämie und für die Aufrechterhaltung einer normalen Blutbildung ist auch das Vorhandensein eines exogenen Faktors (extrinsic factor«) erforderlich. Letzterer ist aller Wahrscheinlichkeit nach im Komplex des B-Vitamins (Hämogen) enthalten. Zusammen mit dem endogenen Faktor bildet das Hämogen das Anahämin, das der wirksame Bestandteil unserer gewöhnlichen Leberpräparate ist. Der endogene Faktor entsteht im untersten Teil des Magens und in den obersten Teilen des Duodenums. Der exogene Faktor ist in unserer gewöhnlichen Nahrung enthalten. Das Anahämin wird sofort zur Blutbildung verwendet; ausserdem wird es aber in mehreren Organen abgelagert, von denen die Leber das wichtigste Depôt ist. Mehrere Beobachtungen scheinen indessen zu zeigen, dass das Anahämin in der Leber nicht als solches abgelagert wird, sondern in Form von Hämogen, das seinerseits durch Fermentwirkung (Hämogenase) in wirksames Anahämin übergeführt wird. Absolut frische Leber ist relativ arm an Anahämin, während autolytierte Leber reich daran ist, da die Autolyse die Bildung von Hämogenase befördert. Zur normalen Blutbildung ist demnach sowohl der endogene wie der exogene Faktor nötig.

Im adulten Leben ist die megaloblastische Blutbildung absolut spezifisch und pathognomonisch für die perniziöse Anämie, wenn man von den megalozytären Spruce- und Botriocephalusanämien absieht. Dies gilt ganz allgemein von den gewöhnlichen Anämien, die man in unserer geographischen Breite antrifft. In subtrophischen und tropischen Ländern kommen auch andere megalozytäre Anämien vor, deren Entstehung man hauptsächlich dem Fehlen eines Typus des B-Vitamins zuschreibt. Zuweilen kann eine schwere Graviditätsanämie gewisse Ähnlichkeiten mit einer echten perniziösen Anämie zeigen.

Während des Embryonallebens dagegen ist vor dem 3. Fetusmonat eine megaloblastische Blutbildung die Regel, und die Erythropoese hat denselben Charakter wie bei der perniziösen Anämie. Diese Ähnlichkeit hat ältere Hämatologen veranlasst, die Blutbildung bei perniziösen Anämie mit dem Ausdruck »Rückschlag zur embryonalen Blutbildung« zu charakterisieren, ein Vergleich, der nach moderner Auffassung in mehrfacher Beziehung stark hinkt. Warum die megaloblastische Blutbildung gerade nach dem 3. Fetusmonat in eine normoblastische Blutbildung übergeht, ist Gegenstand lebhafter Diskussion gewesen. Man hat dies damit in Zusammenhang bringen wollen, dass in diesem Monat sowohl die Magen- und die Intestinalschleimhäute als auch die Leber angelegt werden, Organe, von denen man also weiss, dass sie im adulten Leben den endogenen Faktor bilden. Wahrscheinlich ist das Problem nicht so einfach. Von der Mutter geht sicher der endogene Faktor auf den Fetus über, und die Plazenta selbst enthält reichlich Anahämin. Wichtiger scheint hier die Theorie von der Wachstumshemmung zu sein, die übrigens wahrscheinlich

auf die Entstehung der megaloblastischen Blutbildung bei der perniziösen Anämie anwendbar ist.

Damit sind die wichtigsten der die Erythropoese unter embryonalen und adulten Verhältnissen regulierenden Faktoren besprochen.

Die nun folgende Diskussion über das Megaloblastenproblem bei der perniziösen Anämie fusst auf dem Stadium folgender Punkte:

1. der prozentualen Verteilung der erythropoetischen Zellen im Knochenmark;
2. der peripherischen Retikulozytose,
3. der Grösse der zirkulierenden roten Blutkörperchen ausgedrückt durch ihren mittleren Durchmesser, und der Anisozytose, und
4. der Grösse der peripherischen Erythrozyten, ausgedrückt durch ihr Volumen.

Ad. 1. Das Wesentlichste bei der perniziösen Anämie ist die megaloblastische Blutbildung. Diese pathologische Blutbildung geht unter dem Einfluss der zugeführten Leber mehr oder weniger schnell in die normale, normoblastische Erythropoese über. Durch die grundlegenden Arbeiten von Ehrlich und Naegeli ist dieser ganz charakteristische Typus der Blutbildung bei perniziöser Anämie wohlbekannt. Ihre Untersuchungen wurden später vollständig bestätigt durch die Einführung der intravitalen Knochenmarkuntersuchung mittels der Sternumpunktion. Mit dieser einfachen Methode können wir, selbst von einem Tag zum andern, in allen Einzelheiten die Veränderungen studieren, die in den einzelnen Stadien der Remission im Knochenmark vor sich gehen. Das Prinzipielle bei diesen Veränderungen besteht darin, dass die typische und pathologische Blutbildung rasch verschwindet (zuweilen schon 24 Stunden nach dem Einsetzen der Lebertherapie), um einer anfänglich hyperaktiven und normoblastischen Erythropoese Platz zu machen.

Diese Transformierung von Megaloblastose in Normoblasten bildet den springenden Punkt des Problems. Wie ist es möglich, dass diese Transformierung so ausserordentlich rasch vor sich geht, und wo bleiben die Megaloblasten? Sicherlich zerfallen sie nicht, denn in diesem Falle würde man Anzeichen einer erhöhten Hämolyse in Form eines gesteigerten Meulengrachtwertes und einer vermehrten Urobilinausscheidung im Harn finden, was jedoch nicht der Fall ist. Dieses Problem hat daher von neuem die alte Streitfrage über die Erythrozytengenese wachgerufen. Hier widersprechen sich die dualistischen und die unitaristischen Anschauungen. Naegeli und die meisten seiner Jünger vertraten kategorisch die Ansicht, dass die megaloblastische Blutbildung von der normoblastischen ganz getrennt sei, und dass demnach die megaloblastische niemals ein normales rotes Blutkörperchen bilden könne. Diese dualistische Anschauung hat sich freilich noch bis in die neuere Zeit hinein behauptet. Im grossen ganzen darf man aber sagen, dass nunmehr die unitaristische Theorie, die zuerst

von Maximow und Schilling aufgestellt wurde, allgemein anerkannt ist. Seine vornehmste Stütze erhält der Unitarismus durch die Resultate, die durch die Methode der intravitalen Untersuchung des Knochenmarkes gewonnen wurden. A priori ist es auch nahezu die einzige denkbare Möglichkeit, wenn man den raschen Remissionsverlauf bei der perniziösen Anämie berücksichtigt. Die unitaristische Anschauung wird in vorzüglicher Weise charakterisiert durch ein Zitat von Bock und Malamos: »Die Megaloblastengruppe stellt nur ein krankhaftes Anfangslied in der Entwicklung der Erythropoese dar«.

Wenn ich soeben angeführt habe, dass der Unitarismus seine wichtigste Stütze in der Knochenmarkuntersuchung gefunden hat, so ist dies vielleicht eine Wahrheit, die der Modifikation bedarf. Objektiv morphologisch ist dieser Übergang von Megaloblasten zu Normoblasten nicht festgestellt. Auf Grund meiner eigenen Erfahrung wage ich kaum zu behaupten, dass ich Übergangsformen gefunden habe. Ich habe indessen schon früh darauf aufmerksam gemacht, dass man bei Remission im Mark kernhaltige rote Blutkörperchen von unbestimmtem Typus, sog. Erythroblasten, findet, die demnach sowohl Megaloblasten als auch Normoblasten oder gar Übergangsformen sein können. Ausserdem scheint es fast unmöglich, dass das multipotente und relativ spärliche Reticulum im Mark binnen einer so kurzen Zeit, wie sie hier in Betracht kommt, im Stande sei eine ganz neue Generation von roten Blutkörperchen zu bilden. Schon rein ziffernmässig ist dies eine Unmöglichkeit. Auch morphologisch fehlen Anzeichen einer stark gesteigerten Aktivität von Seiten des Reticulums in Form von Mitosen und Amitosen. Die explosionsartige Bildung von Normoblasten deutet darauf hin, dass sich die Megaloblasten teilen und Normoblasten bilden. Morphologische Erscheinungen, die als Stütze hierfür dienen können, sind auch nachgewiesen von Lisa Boström (noch nicht veröffentlicht) beim Studium einiger perniziöser Anämien mit besonders ausgesprochener normoblastischer Neubildung.

Ad. 2. In dem peripherischen Blute wird der Remissionsverlauf hauptsächlich an der Retikulozytenzunahme studiert. Es ist wohlbekannt, dass die Retikulozytenspitze erst später kommt als die normoblastische Verwandlung der Megaloblastose im Knochenmark. Vorausgesetzt, dass die Leberzufuhr optimal war, kann man damit rechnen, dass das megaloblastische Knochenmark am 2. bis 5. Tage ganz verschwunden ist. Unter der gleichen Voraussetzung entsteht das Maximum der Retikulozytenzunahme im peripherischen Blute während des 5. bis 8. Tages. Im grossen ganzen gilt, dass die Höhe der Retikulozytenspitze dem Grade der Anämie wie auch dem Grade der Megaloblastose der Knochenmarks proportional ist. Erst nachdem die Retikulozytose abzunehmen begonnen hat, erfolgt die Vermehrung des Hämoglobins und der roten Blutkörperchen.

Ad. 3. Grundlegend für jedes Studium des mittleren Durchmessers der roten Blutkörperchen sind die nun bald klassischen Kurven von

Price-Jones. Bei der unbehandelten perniziösen Anämie ist der mittlere Durchmesser der Erythrozyten wesentlich vergrößert und beläuft sich auf etwa $8,5\mu$. Indessen wird sehr oft das sehr Charakteristische in der Grössenstreuung der roten Blutkörperchen übersehen. Typisch und pathognomonisch für die perniziöse Anämie ist die sehr erhöhte Streuung. Diese Anisozytose kann bis zu 8 bis 9μ betragen. Während der Remission beobachtet man eine sehr wichtige Erscheinung, dass nämlich der mittlere Durchmesser etwas zunimmt, um später auf die normalen Werte zurückzugehen. Diese Normalisierung der Grösse des mittleren Durchmessers erfolgt zufolge den meisten Verfassern ausserordentlich rasch. Wenn es sich um die Anisozytose handelt, findet man, dass nach den Untersuchungen der meisten Verfasser die Streuung während der Remission rasch abnimmt. Man muss jedoch hinzufügen, dass die auf der Bestimmung des Durchmessers der roten Blutkörperchen beruhende Untersuchungsmethode recht unsicher ist und daher viele Fehlerquellen birgt. Die Megalozyten sind ja in der Regel Ovalozyten, weshalb es eine einfache Bestimmung des grössten Durchmessers nicht genügen dürfte, obgleich man es doch gewöhnlich dabei gewenden lässt.

Ad. 4. Grössere Möglichkeiten bietet dagegen die Bestimmung des Durchschnittsvolumens der roten Blutkörperchen. Durch die Bestimmung des Hämatokritenwertes erhält man leicht das Durchschnittsvolumen. Die Arbeiten Bönningers sind hierbei grundlegend. In der Regel ist bei der unbehandelten perniziösen Anämie eine bedeutende Vergrösserung des Durchschnittsvolumens der Erythrozyten nachzuweisen das 100 bis $110\mu^3$ beträgt. Während der Remission steigt das Durchschnittsvolumen anfangs bis zum »Stadium der maximalen Zellenvergrösserung«. Dieser Anstieg des Volumens der Erythrozyten scheint der Zeit nach mit der Vermehrung der Retikulozyten zusammenzufallen. Hierauf erfolgt ein allmählicher Rückgang zum normalen Zellenvolumen.

Eigene Untersuchungen.

Das Material umfasst 6 perniziöse Anämien, die in bezug auf die Knochemark- und Blutveränderungen in den erwähnten Beziehungen ausführlich studiert wurden. Die Diagnose war in sämtlichen Fällen ganz klar. Die Fälle wurden in einem Zeitraum von 20 Tagen studiert. In sämtlichen Fällen wurde die Leberzufuhr als optimal angesehen, und in der Regel wurde Pernaemon forte oder Heptomin in Injektion in einer Dosis von insgesamt 12 ml an drei auf einander folgenden Tagen (in der Regel am 2., 3. und 4. Tage nach der Aufnahme des Patienten ins Krankenhaus) administriert. Die Bestimmung des mittleren Durchmessers wurde »mit der nassen Methode« ausgeführt. Der Hämatokritwert wurde mit Doppelprobe bestimmt. Es wurde möglichst dieselbe Zählkammer zum Zählen der Anzahl der roten Blut-

Tabelle I.

Die Variationen des Hämoglobins und der roten Blutkörperchen in sechs Fällen von perniziöser Anämie.

	Fall 1		Fall 2		Fall 3		Fall 4		Fall 5		Fall 6	
	Hbl %	R. Blkr. milj.	Hbl %	R. Blkr. milj.	Hbl %	R. Blkr. milj.	Hbl %	R. Blkr. milj.	Hbl %	R. Blkr. milj.	Hbl %	R. Blkr. milj.
0	54	1,94	45	2,15	29	1,20	52	1,78	38	1,58	60	2,85
1												
2			42	2,16					38	1,56		
3	53	1,90			24	1,10					70	3,17
4							50	1,99	35	1,38		
5	48	1,80	51	2,38			53	2,02	36	1,62		
6												
7	56	1,88			25	1,07			39	1,69	71	3,15
8							55	2,38				
9	62	2,77	48	2,24					45	2,16		
10												
11	65	2,80	57	2,52	27	1,08	62	2,77	48	2,37		
12												
13					32	1,40			56	2,78	81	3,58
14			61	2,88			63	2,58	62	3,13		
15	63	3,08			44	2,00						
16									56	2,93		
17					56	3,05	62	2,58	58	3,26	81	3,61
18			68	3,40	64	3,04			62	3,24		
19												
20	70	3,50					65	2,62	63	3,29	80	3,82

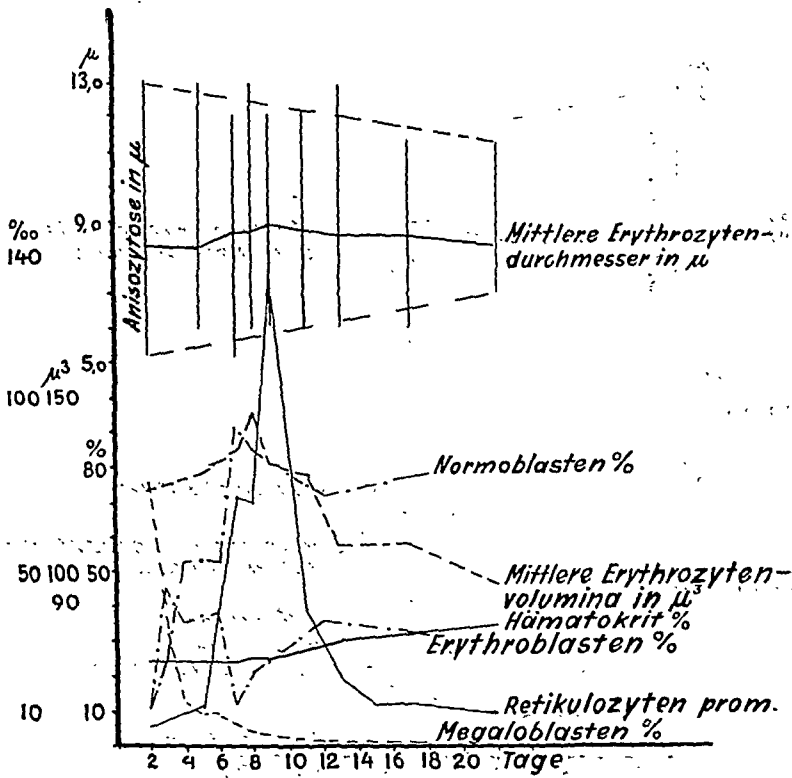
körperchen und so weit möglich in jedem einzelnen Fall benutzt, um die Methodenfehler in grösstmöglichem Umfange zu neutralisieren.

In der folgenden Tabelle sind die Blutwerte in Bezug auf Hämoglobin (Prozente) und rote Blutkörperchen (Millionen) zu finden. Die Tafeln (I—VI) geben im Blut die Variationen der Retikulozyten (Promille), Hämatokrite (%), des Durchschnittsvolumens (μ^3), des mittleren Durchmessers und der Anisozytose (μ) und im Knochenmark die Variationen der Megaloblasten, Erytroblasten und Normoblasten (%) wieder.

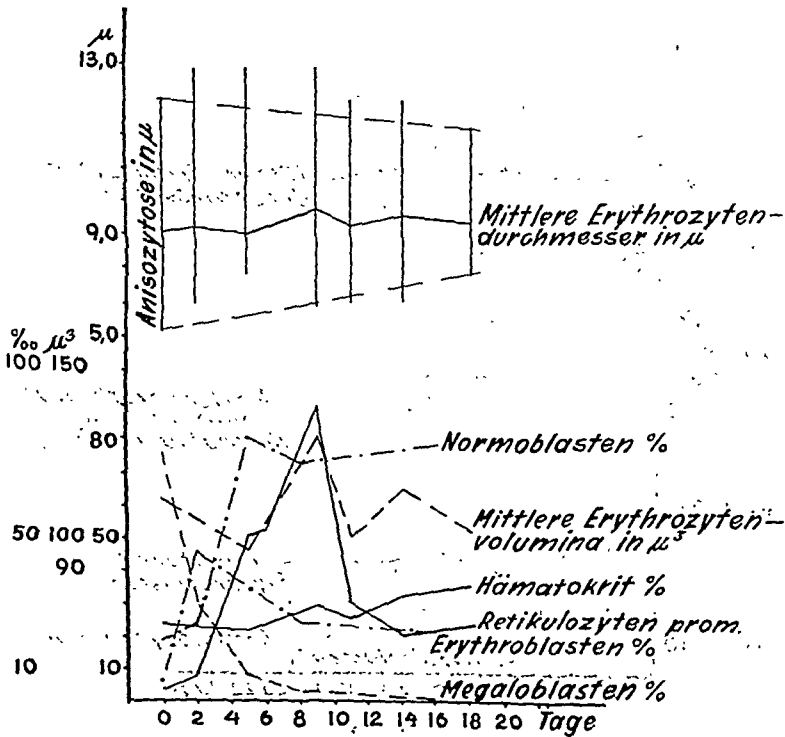
Ergebnisse.

Im Prinzip erhält man in sämtlichen Fällen dieselben Veränderungen der studierten Details. Im peripherischen Blut kommt die Retikulozytenspitze etwa 8 Tage nach der Leberzufuhr. Gleichzeitig mit dieser Spitze erscheint eine vorübergehende Steigerung des mittleren Volumens und des mittleren Durchmessers. Die Anisozytose nimmt sukzessive ab, aber der mittlere Durchmesser ist noch nach 20 Tagen bedeutend vergrössert.

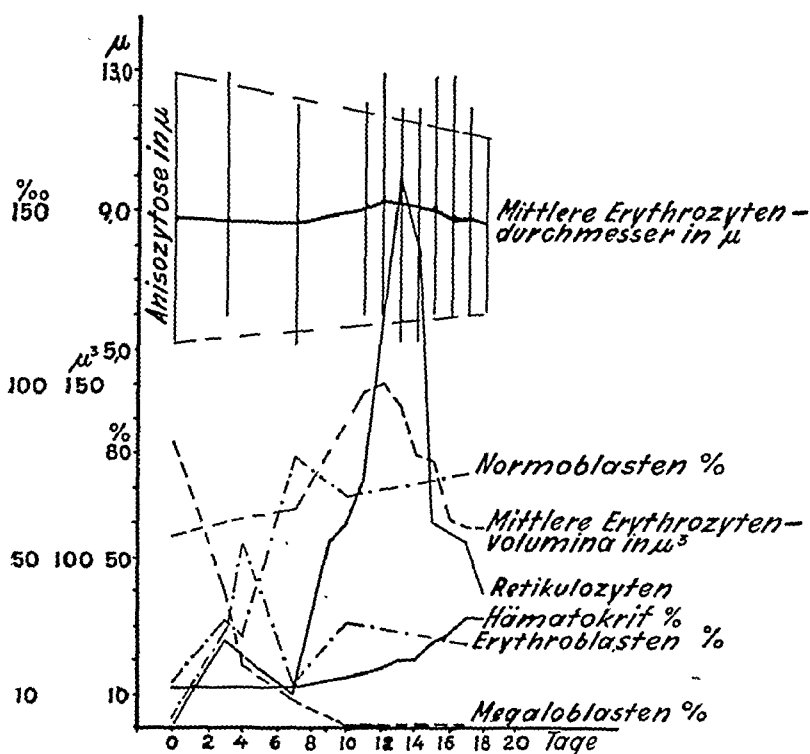
Im Knochenmark verschwinden die Metaloblasten ausserordent-



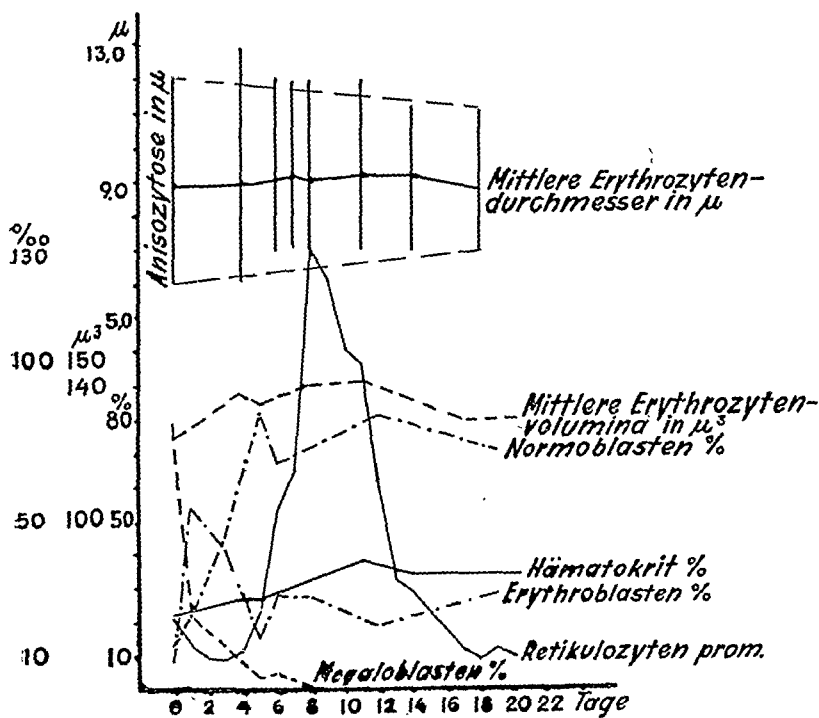
Tafel I.



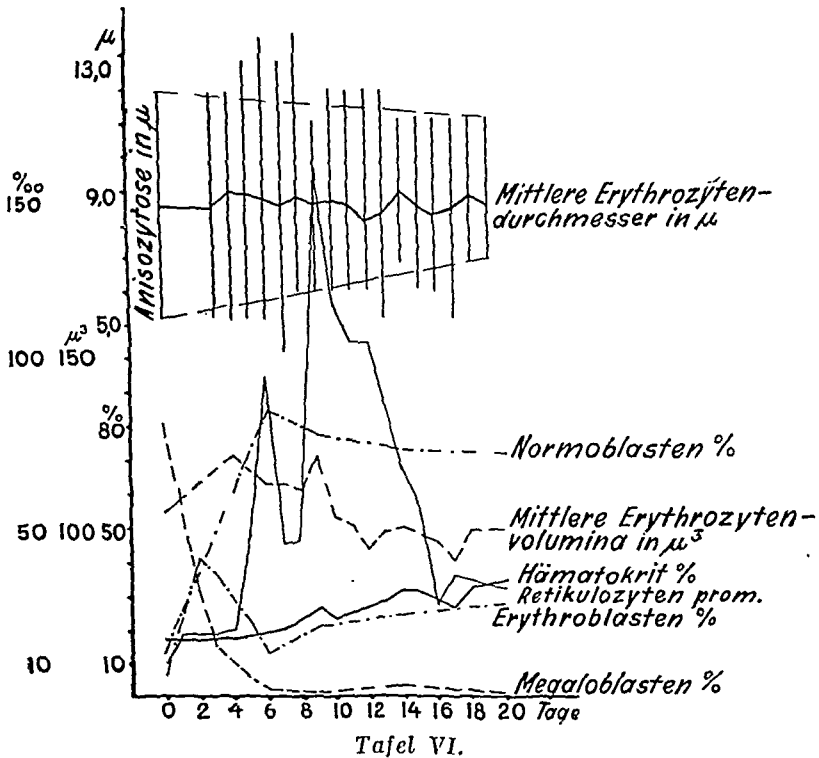
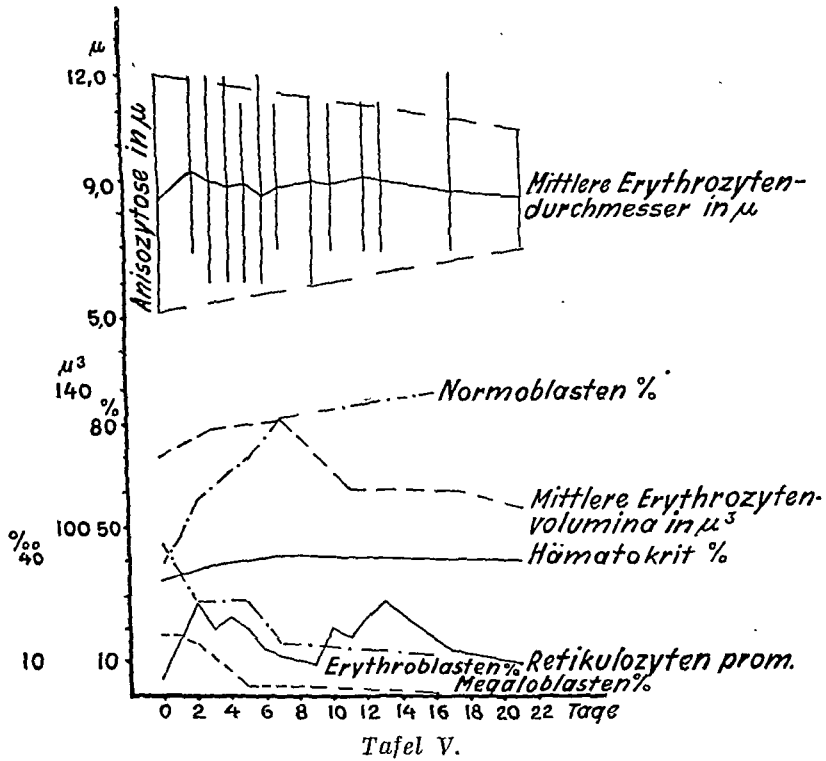
Tafel II.



Tafel III.



Tafel IV.



lich rasch, einzelne Exemplare sind aber noch am 20. Tage vorhanden. Gleichzeitig mit dem Verschwinden der Megaloblasten erfolgt eine Vermehrung der Erythroblasten als Normoblasten. Unter den Erythroblasten sind hier kernführende rote Blutkörperchen zu verstehen, deren Morphologie bald mit Normoblasten bald mit Megaloblasten gewisse Ähnlichkeiten aufweist. Diese Vermehrung geht der Retikulozytenspitze im peripherischen Blut voraus. Noch am 20. Tage ist die Zunahme dieser Zellenart vorhanden.

Diskussion und Kommentare.

Die gewonnenen Ergebnisse stimmen mit den Untersuchungen aus der Literatur vollständig überein. Kann nun die Untersuchung irgendwie zur Lösung des Megaloblastenproblemcs beitragen? Sehen wir uns zuerst die einzelnen Details an, so finden wir, dass die Retikulozytenspitze, die gewissermassen als pathognomonisch für die Remission der perniziösen Anämie angesehen wird, im grossen ganzen dem Grade der Megaloblastose im Mark proportional ist. Mit andern Worten: die Voraussetzung, dass eine Retikulozytenspitze von diesem Typus zustandekommt, ist das Vorhandensein einer Megaloblastose. Wenn diese bei der Remission verschwindet, ist auch keine erneute Retikulozytose nachzuweisen, auch nicht nach erneuter Administration von Leber. Diese wohlbekannte Tatsache findet demnach ihre volle Erklärung.

Die gleichzeitige und vorübergehende Vergrösserung des mittleren Durchmessers wie des durchschnittlichen Volumens ist wohl dadurch zu erklären, dass die Leber wenigstens auf einen Teil der Megaloblasten im Mark ausreifend wirkt. Die Retikulozyten sind daher Makrozyten, was Bock und Malamos durch Messungen nachgewiesen haben. Der überwiegende Teil der Megaloblasten kann jedoch nicht auf diese Weise ins Blut übergehen, da man nie eine, wie man in diesem Falle erwarten sollte, unmittelbare Vermehrung der roten Blutkörperchen findet.

Man muss sich denken rein physiologisch dass eine Transformation in die ebenerwähnten Erythroblasten erfolgt, die demnach Nachkommen der Megaloblasten bilden. Sehen wir uns die Kurven an, so zeigt sich, dass vor der kräftigen Normoblastenvermehrung eine Vermehrung der Erythroblasten erfolgt. So lange die Normoblastenvermehrung anhält, sind auch Erythroblasten vorhanden. So weit ich sehe, müssen diese Erythroblasten demnach die Übergangsform von der pathologischen megaloblastischen zur normalen normoblastischen Blutbildung darstellen. Die schwierige Frage des Unitarismus oder Dualismus der Erythropoese scheint mir auf akzeptable Weise gelöst zu sein. Der Unterschied zwischen den Megaloblasten und Normoblasten besteht in dem ungleichen Reifegrad, und unter der Einwirkung

der Leber bilden sich die Normoblasten aus den Megaloblasten auf dem Wege über die Erythroblasten. Für diese Deutung spricht ferner, dass auch im völlig normalen Mark Promegaloblasten oder richtiger Proerythroblasten vorkommen, die morphologisch nicht von den Promegaloblasten zu unterscheiden sind, welche man bei perniziöser Anämie im Mark findet, und die ihm sein charakteristisches Aussehen verleihen. Diese Deutung macht das äusserst rasche Verschwinden der Megaloblasten bei der Remission weniger rätselhaft.

Zusammenfassung.

Bei der Remission der perniziösen Anämie lassen sich verschiedene Phasen erkennen. Eine Phase, die relativ schnell verläuft, besteht im Ausreifen von makrozytären Retikulozyten von Megaloblasten, wobei die Retikulozyten im Blut die sichtbare Folge der beginnenden Remission sind.

Eine zweite Phase ist die Transformierung der Megaloblasten in Normoblasten über die Erythroblasten. Diese Phase verläuft anfangs äusserst rasch, später aber langsamer. Im Blute spiegelt sich der schnellere Teil der Phase in der Zunahme des mittleren Volumendurchmessers und die spätere mehr langsame Phase in einer recht lange anhaltenden Makrozytose wider.

Die Normalisierung der Makrozytose erfolgt bedeutend langsamer als nach den Angaben der Literatur. Die Anisozytose dagegen nimmt relativ schnell ab.

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A CASE OF METASTATIC HEART TUMOR — NEUROFIBROSARCOMA

By *Olav Anthun.*

(Received for publication 4. 3. 1943.)

Neurofibrosarcoma is hardly of rare occurrence. The case presented here has offered several features of interest, as tumor metastases were distributed to the heart and not recognised while the patient was alive. No definite clinical diagnosis could be made; the microscopic examination was decisive.

The patient is a woman, 61 years old. The history of her illness is briefly as follows: 25 years ago, fractura cruris. 10 years ago operated for uterine tumor. In January 1940 she had fractura colli femoris. 2 years later she returned for control examination of the hip. She had had no trouble from the fracture in the hip. She did not feel well, however, she had a cold and had lost weight, and for these reasons she was admitted to the Medical Department 23/3-42. She told that 6 years earlier she had had hypertonia, styes on the eye-lids and infected tumors in the scalp, for which she had been treated with autovaccine and had recovered. Last winter, cough and expectoration. 6 weeks previous to admission she felt tired and weak, had headache on left side and pain in back of the neck and down the right shoulder and arm. She also felt pain along the ulnar margin of the forearm and the 2 ulnar fingers. The entire arm and the fingers were without strength. Temperature slightly subfebrile. She had to stay in bed because of exhaustion. On examination the patient appeared pale. Temperature 37.9. Pulse 92, regular. Blood pressure 130/80. On the scalp were seen 6 pea-sized, solid, easily moveable, non-sensitive tumors. No enlargement of the cervical glands. Normal finding over the lungs. Ictus of the heart in the 5' intercostal space, inside the pappillary line. Sharp systolic murmur over the 2' left intercostal space; action regular.

The following special examinations were made: Sedimentation rate 23 mm., Wassermann negative in the blood. Blood urea 50 mg.%. Hb. 82 %. White blood cells 6400. Differential count revealed nothing special. Plasma color 3. Ewald test meal: Congo 0; total acidity 0/15; Uffelmann 0. Roentgen examination of stomach: Peasized, light area in bulbous duodeni, which was interpreted as a polyp. Roentgen examination of the lungs showed scattered

throughout both lungs, especially on right side, numerous almond-sized, fairly dense infiltrations of typical tumor appearance. 30/3, Electrocardiogram showed regular sinus rhythm, frequency 100, P. less than 0.10, PQ. 0.16, QRS. 0.06. S-T line positive, T 1 negative, T.2 isoelectric, T 3 positive. Diagnosis: Myocardiopathy.

On 2/4, it says in the case record, 3 moveable skin-tumors of various sizes and of the same appearance were found, 2 anteriorly on the chest and a larger one posteriorly on right flank.

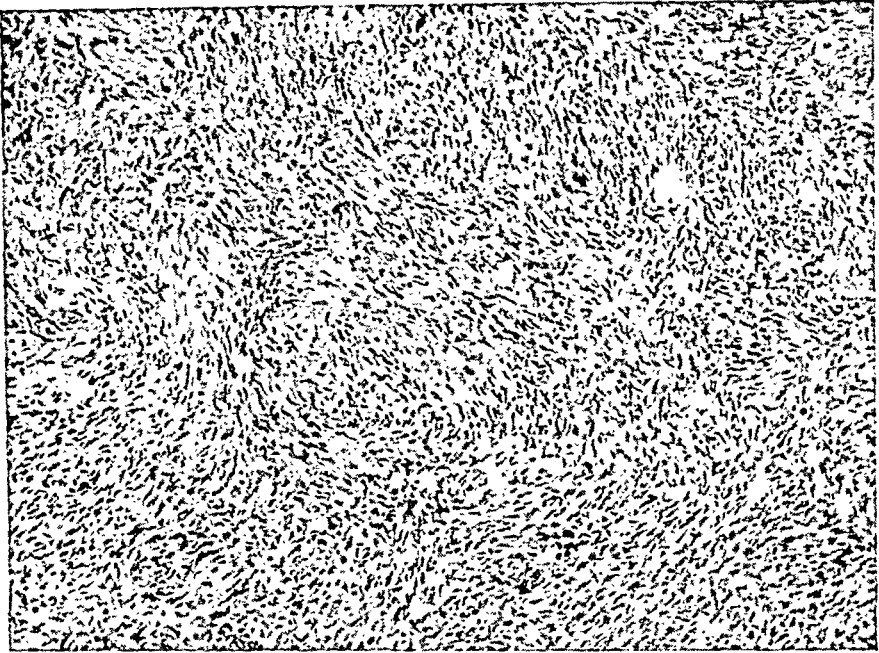


Fig. 1.

Tumor tissue from the extirpated skin-tumor. Magnif. $\times 100$.

This tumors had not been observed on admission, and they probably developed during the patient's stay in hospital.

The criteria for a diagnosis were the Roentgen findings, consisting of a polyp in the bulbus duodeni and tumor metastases to the lungs. The other examinations gave no information as to the site of the primary tumor. Attention was then drawn to the skin tumors found on the chest and abdomen. On 14/4 one of these tumors was extirpated for microscopic examination. The report read:

In the corium and subcutaneous adipose tissue a vaguely defined tumor tissue is found. It is made up of spindle-shaped cells arranged in irregular bundles and streaks, indistinctly outlined from the surroundings. There is a moderate variance in the nuclear size and chromatin content, and a few mitoses; there is a tendency to wreath arrangement, but no convincing palisade nuclear arrangement. The stroma is moderately rich in cells, with coarse fibers, and contains scattered lymphocytes. Some normal appearing

sweat glands are found. No epithelial tissue is seen and the picture suggests: *Fibroma or neurofibroma, rich in cells.* (Eker.)

As the biopsy gave no definite evidence of the skin tumor being a sarcoma, these tumors were regarded as accidental coincident phenomena, and the case was no clearer.

The condition of the patient became steadily aggravated, and she died on 18/4.

Autopsy, (only positive findings are reported):

Right lobe of the *thyroid gland* is occupied by a solid tumor with greyish-white sectional surface, hard as cartilage and as large as a plum. Left lobe usual appearance, hard, with a greyish-white sectional surface. No enlarged lymph-nodes on the neck.

Pericardium. Abundant fluid on opening of the pericardial cavity; the walls are smooth.

Heart, fairly large. Scattered over epicardium are numerous hard, greyish-white nodes with compact sectional surface, from hemp-seed to peasize. Some are distinctly prominant, none stalky. On opening of the heart, vaguely delined tumor infiltrates it are seen in the right and left ventricular walls. The myocardium of both ventricles is tumor-infiltrated and decayed; endocardium smooth and moist. Not abnormality of the valves, ostia and auricles. The walls of the coronary arteries are smooth; no constriction of lumen.

Pleura. Small amounts of fluid in left pleural cavity and easily detachable adhesions at base of the lung.

Lungs. Scattered throughout both lungs are nodes similar to those seen in the heart, sizes from pea to hazel-nut. The hilus glands are moderately enlarged, some are of the same consistency and appearance as the tumor-nodes in the lungs.

Peritoneum. Small amounts of ascites fluid. In peritoneum parietale, corresponding to both inguinal regions, there are a few subserous flat tumor nodes.

Liver interspersed throughout with nut-sized nodes.

Pancreas visibly enlarged; up to nut-sized nodes are scattered throughout, making the entire organ hard and infiltrated.

Kidneys. Underneath the capsule a couple of nodes, from grains to hemp-seeds in size.

Stomach and intestines. The stomach is studded with similar tumornodes, partly subserously, partly submucously. In bulbus duodeni there is a walnut sized tumor node. Also in the intestinal canal there are numerous flat, well defined, pea-sized nodes, mostly localised to the colon.

Adrenals, spleen and genitalia interna: No abnormality.

Microscopic Examination. Sections from a new-formed *subcutaneous node* from dorsum of thorax presented essentially the same picture as the extirpated tumor. In sections from the *thyroid gland*, the thyroid tissue in the chosen section was practically entirely replaced by tumor tissue, which appeared as islands and streaks separated by bands of connective tissue, that partly was hyalinised, partly necrotic. The tumor-cells were polygonal, somewhat varying in appearance in the different parts. In some places the cells were spindle-shaped and arranged in bundles, in others there was a perivascular arrangement of the tumor tissue. The cells were only slightly differentiated, with irregular or round nuclei varying in size and chromatin content. In one place there was accumulation of giant cells, of the foreign body type. The stroma was somewhat infiltrated by leucocytes. In sections

from the left lobe of the thyroid gland the thyroid tissue was better preserved. It was diffusely infiltrated by tumor tissue, however, and the cells were mostly spindle shaped and arranged in irregular bundles. In one place there was a tendency to palisade nuclear arrangement. In sections from *the lungs* the atypical tumor tissue was recognised as an indistinctly outlined node. The cells here were partly spindle shaped, partly more polygonal. There was no definite palisade nuclear arrangement, but wreath formations in places. In sections from *the liver* a node of the same tumor tissue was identified. Also here polygonal and spindle shaped cells were alternating. In sections from *the kidney* a node of the same tumor tissue was found, situated immediately inside the capsule. The cells were partly polygonal, partly spindle-shaped. In sections from the *pancreas* the glandular tissue in the chosen sections, was to a great extent destroyed. Diffuse infiltration of the already described tumor tissue was widely distributed. Also here bundles of spindle-shaped cells were noticed between the polygonal tumor cells. The cells in the islands of Langerhans were relatively well preserved. Diffusely distributed infiltration of lymphocytes and plasma cells was noticed in the stroma. In sections from *the musculature of the heart*, left ventricular wall, the musculature was found diffusely infiltrated by the same tumor tissue. The cells were to a great extent spindle-shaped. Several hemorrhages and areas of necrosis were seen.

The findings in the various organs spoke decidedly in favour of the presence of neurofibrosarcomatosis with multiple metastases to the internal organs. After renewed study of the biopsy on the skin node, I believe that the cellular atypia is distinct, and the structure of the tissue is so characteristic that the diagnosis of neurofibrosarcoma may be made. The tumor tissue in the internal organs is less differentiated, but also here the spindles-shaped cells were arranged in bundles and wreaths. The only differential diagnosis that otherwise might come into consideration was polymorphocellular sarcoma of the thyroid gland. There is some divergence however, as to whether primary sarcomas do occur in the thyroid at all. Ewing claims that the polymorphocellular sarcoma is a slightly differentiated carcinoma, in which the alveolar structure may be recognised, and that metastases show the same structure as the primary tumor.

This peculiar case of ours has not been fully explained in spite of the reports on the microscopic findings. There was the information that 6 years previously the patient had »infected« tumors in the scalp. The infection soon receded. Microscopic examination of these tumors had not been made. Later she had noticed these tumors no more. They were pointed out on admission to the Medical Department. During the stay in the hospital several similar nodes appeared in the skin of the chest and abdomen. No information of pigmentations of the skin or familiar occurrences of neurofibromatosis was obtained. It might be considered whether this was an atypical case of v. Recklinghausen's disease with »malignant degeneration« and metastases. Gabel distinguished between fully developed and abortive forms of v. Recklinghausen's disease. Harbitz found that incomplete forms do occur, and that the picture is blurred by development of

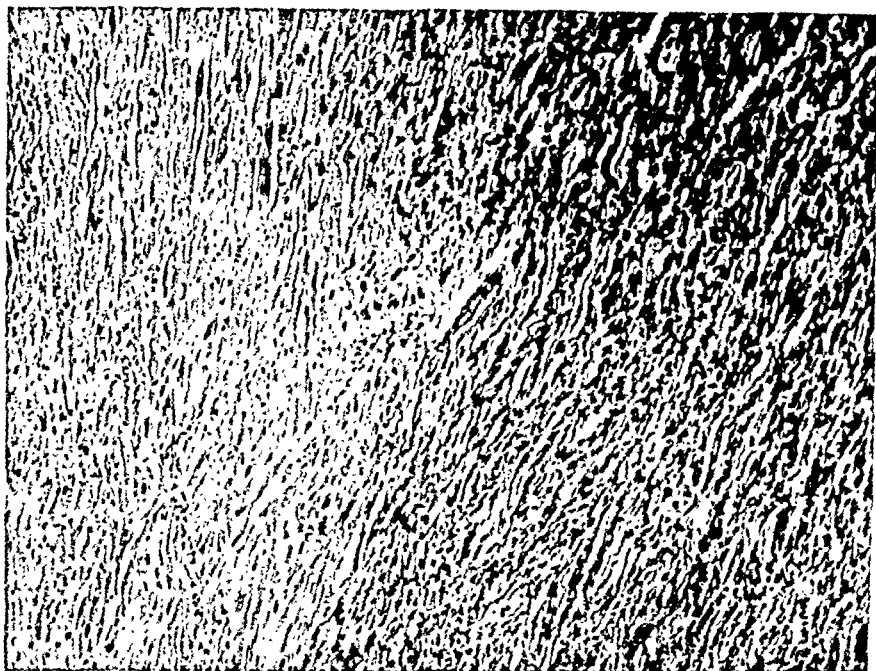


Fig. 2.
Tumorinfiltration in the heart. Magnif. $\times 100$.



Fig. 3.
Tumorinfiltrated heart.

»malignant degeneration«. Dubois described as v. Recklinghausen's disease a case which presented only a couple of skin tumors and a bulky mediastinal tumor of neurinomatous structure. Likewise Charrierre and Huriez had 2 cases of mediastinal tumors, the first one with, the second without skin tumors.

What today is known of v. Recklinghausen's disease, is that it develops in the course of decades and that as a rule the patients do not die from this, but from an intercurrent disease. Deaths consequent to »malignant degeneration« are by most authors given to be about 12 % (Harbitz, Jackson, Herxheimer and Roth, Stewart and Copeland). In the literature I have found that relatively few of these cases were examined post mortem. Of 34 cases Harbitz had 2 certain neurofibrosarcomas, none of which was autopsied. Other authors found metastases to the lungs, mesenteric and retroperitoneal lymph-nodes, adrenal medulla, subserous nodes in stomach and intestines, diaphragm, pancreas, liver and skeletal system (Herxheimer and Roth, Jackson, Palmstierna, Heine, Borroni, Tramantano e Pansini, Ewing, Stewart and Copeland). I have found no mention of metastases to the heart.

Our patient died essentially from cachexia. She offered no subjective symptoms from the heart. An electrocardiogram taken 18 days before she died showed, as already mentioned, only slight myopathic changes and no disturbance in nervous conduction. Thus, this was a metastatic tumor infiltration of the heart. Mönckeberg and Ribbert have reviewed the literature on this subject and found that carcinomas as well as sarcomas do occur in this heart. As to metastatic heart sarcomas, metastases have previously been described from melanoma, humerus-sarcoma and sarcoma in the region of the hip, subphrenic spindle-shaped sarcoma and lymphosarcoma of the intestine. All these showed diffuse infiltration of musculature of the heart, besides epi- and endo-cardial nodes. Geipel reported a case of myxosarcoma in the abdominal wall with metastases to the heart. This did not cause disturbances in nervous conduction in spite of extensive tumor infiltration. The present case, in the same way, demonstrates that considerable tumor infiltration of the heart may occur without giving clinical evidence and without marked electrocardiographic changes.

Summary.

The author reports the case of a woman, 61 years old, who the last 6 years had had some skin tumors in the scalp without suffering any inconveniences herefrom. After 5½ years, fatigue and exhaustion developed, she lost weight and was admitted to a hospital without any definite diagnosis having been made. During the stay in the

hospital more skin-tumors developed on her body, one of which was extirpated for examination, and the diagnosis of neurofibroma was made. Polyp of the bulbus duodeni was demonstrated roentgenologically, likewise tumor metastases to the lungs. She died of cachexia without the primary site of the malignant tumor having been discovered. Autopsy revealed multiple tumor metastases to a number of internal organs, including the heart. Microscopic examination disclosed neurofibrosarcoma. The author discusses whether or not this case is atypical v. Recklinghausen's disease with »malignant degeneration«. Tumor infiltration in the heart in this disease has not been reported previously. The tumor infiltration was very considerable, and yet it gave neither clinical symptoms nor marked electrocardiographic changes.

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THE POSITION OF FUSOBACTERIUM AND LEPTOTRICHIA IN THE BACTERIOLOGICAL SYSTEM

By *Johs. Bøe* and *Th. Thjötta*.

(Received for publication May 10th 1943.)

The international committee on bacteriological nomenclature and on the arrangement of the bacteria in a logical system has done a very usefull work and has brought about a better order in the numerous families, genera and species of bacteria than hitherto has been the case. The system and the new nomenclature is to be found in the fifth edition of Bergey's Manual of Determinative Bacteriology.

It cannot, however, be denied that the said committee, following older descriptions of bacteria, in some cases has placed certain bacteria in families where they do not belong and has separated bacteria that obviously are very closely related. In the following paper we will discuss such a case, namely the systematic position of the *Leptotrichia* and the *Fusobacterium*.

Every bacteriologist will be aware of the fact, that the nonsporulating anaerobes and their relation to one another are very poorly known. Some of them are studied quite closely, while others have been dealt with only occasionally and often been misunderstood. Consequently it is necessary to take this question up for discussion. We do this as regards the Genera *Fusobacteria* and the *Leptotrichia* since we have been interested in these bacteria and have studied both genera quite thoroughly (1, 2) through some years.

I. *Leptotrichia*.

The description of the *Leptotrichia* of the mouth goes back into the very childhood of bacteriology and was originally based only upon morphological characters, as the name indicates («*Leptotrichia*» = a thin hair). It will not be necessary here to deal with the descriptions given by *Friedrich Kützing*, *Friedrich Buehlmann*, *Charles Philip*

Robin, V. Trevisan and others of an early period. Here we can refer to our previous work on the same subject (1). These authors, like *W. D. Miller*, were satisfied with the morphological study of the microbe, while *K. W. Goadby* made an attempt to cultivate it and study its biochemical characters.

After 1900 we find many papers dealing with *Leptotrichia* in the literature, mainly under the name *Leptotrix* but only a few of these papers are worth mentioning, as the authors very often have contributed more to the confusion than to the elucidation of the subject.

In his work with the bacteria present in caries dentis *I. J. Kligler* (3) studied the *Leptotrichia*. He cultivated 15 strains and gave an accurate description of their cultural and biochemical characters. He states that the individual bacteria of his strains were Grampositive in young cultures, but very soon lost this character as they gained in age. None of his strains produced indol nor ammonium and they did not liquefy gelatin. The microbes were not pleomorphic, but showed often long filaments, that might break up into shorter rods. The growth was anaerobic or facultative aerobic.

Also *W. B. Wherry* and *W. W. Oliver* (4) gave a good description of these microbes, and especially of the colonies of their strain.

In Germany *H. A. Gins* (5) in 1934 studied the *Leptotrichia*, in several papers. He has cultivated many strains and has given an account of their morphology and cultural characters. His strain Gi XXIII especially claims our interest. This strain consisted of rather short rods that were pointed in both ends and looked very much like a *Fusobacterium*. It was called »*Riesenfusiformis*« until its place was found to be among the *Leptotrichias*. Gins, however, does not account for the distinction between these two different genera.

The distinction between *Leptotrichia* and other threadlike microbes is still a problem for many authors, and especially that between the said microbe and the *Fusobacterium* seems to be quite complicated. We feel that many of the recent papers on the *Fusobacterium* deal with strains named as *Fusobacterium* but obviously belonging to the *Leptotrichia*. Thus *Philip L. Varney* (6) in his work from 1927 in his group IV and partly in group III erroneously has described strains of *Leptotrichia* as *Fusobacterium*. This is also the case with the strain described by *Maynard K. Hine* and *George Packer Berry* (7) under the name *Fusiformis dentium* and also with the main part of the strains described by *Earle H. Spaulding* and *Leo F. Rettger* (8) as *Fusobacterium* of type II. The latter authors discuss the possibility that their strains did not belong to the same genus, but the occurrence of intermediate forms made them place them into the same genus as *Fusobacteria*.

Basil G. Bibby and *George Packer Berry* (9) isolated strains that might belong to one genus or to the other, as they found that no bacterial system gives a clear distinction between *Leptotrichia* and

Fusobacterium. They state as their opinion that the system of the threadlike bacteria still is unclear and difficult.

In Germany *Erhard Nieber* (10) isolated some strains of *Actinomyces* and of *Leptotrichia*. He examined, together with his own strains, four strains of *Leptotrichia* from Gins' material and found it difficult to distinguish between these strains and strains of *Fusobacterium*.

In the last edition of *Bergey's Determinative Bacteriology* the *Leptotrichia* are placed in the second order and as the first genus of the family *Actinomycetaceae*. The genus is described, as follows: »Thick, long, straight or curved filaments, unbranched, frequently clubbed at one end and tapering to the other. Gram-positive when young. Filaments fragment into short, thick rods. Anaerobic or facultative. No aerial hyphae or conidia. Parasites or facultative parasites.« Two species are described, *L. buccalis* and *L. placoides*. Both species are said to liquefy gelatin.

We shall describe *Leptotrichia* as follows:

The genus *Leptotrichia* consists of straight or slightly curved rods, mostly about 10 microns in length, and 0.7—1.2 microns in diameter. The length may, however, vary to a great extent. One may find rods of 3 microns and in fluid cultures long filaments of up to 200 microns. As a rule, however, two rods are found together, connected and forming a short filament. The rods are smoothly formed with a slight tapering to the ends. There is no club-formation. Often two organisms are found in a position that very much reminds of a *Fusobacterium* in the typical position of this microbe. The *Leptotrichia* is Gram-positive in very young cultures but it loses this character very soon, and thus the older cultures will show negative rods.

All *Leptotrichia*-strains are anaerobic or microaerophilic microbes. After some generations, however, one may see a sparse growth also in aerobic cultures. The typical colony may be described as follows: It is 1 mm. after 24 hours, and 2 mm. after 48 hours. On account of its build it is easily picked out among the other colonies on the plate. Examined with a hand-lense it has a pearly lustre and shows wavy tufts or locks surrounding the colony and thus giving it the appearance of a Medusa-head. Under the microscope the very young colony is seen to consist of rods or filaments closely packed together like rafts of timber in a river. Colonies such as described are especially seen on media consisting of brain-heart extract. On blood-agar the colonies do not obtain that typical aspect and this medium is not so well suited for picking out the colonies, as it is not transparent. The colonies on blood agar are smaller and more irregular than on brain-heart medium. No hemolysis is seen, but only a weak greenish discoloration.

The biochemical reactions of *Leptotrichia* are distinct and clear-cut. It is an active fermenter of carbohydrates and produces acid

in dextrose enough to lower the p_H of the medium from 7.4 to about 5 (4.69—5.17). No gas is produced, indol is not formed, and nitrate is not reduced to nitrite. Gelatin is never liquefied.

Injected into rabbits *Leptotrichia* makes a good antigen and a complement-fixing antibody is quickly produced in the animals. With several such sera no distinct types of this microbe could be separated, while it was very easy serologically to distinguish between *Leptotrichia* and *Fusobacterium*.

Leptotrichia is not pathogenic. With filtrates from cultures, however, it is possible to obtain the *Shwartzmans* phenomenon in rabbits.

II. *Fusobacterium*.

The classification of *Fusobacterium* is difficult for various reasons, such as the polymorphism and the great adaptation of this microbe to new conditions of growth. The tendency of the authors to create new names for microbes unsatisfactorily studied and described and thus name new species or genera when meeting with strains showing small or inconstant differences from the type also has added to the confusion in the classification of the *Fusobacterium*.

Maximilian Knorr (11) studied four strains of *Fusobacterium* which he placed in one genus *Fusobacterium*. This genus was divided into three types, and this division is often used, although it obviously suffers from the weakness that it is founded on only four strains and even on their morphological appearance.

Knorr tried to classify strains of *Fusobacterium* described earlier by other authors. But this attempt turned out to be a very difficult one, since the various types of the authors did not show conformity with his types.

Also the more recent works on *Fusobacterium* and its classification show that it is difficult or even impossible to find a convenient and logical agreement among the different authors. We shall name the following authors: *Philip H. Varney* 1927 (6), *H. A. Gins* 1928 (12), *David T. Smith* 1932 (13), *Earle H. Spaulding* and *Leo Rettger* 1933 (8), *Lawrence W. Slanetz* and *Leo Rettger* 1937 (14), *M. Weinberg*, *R. Nativelle* and *A. R. Prevot* 1937 (15), *Maynard K. Hine* and *George Packer Berry* 1937 (7), *A. R. Prévot* 1938 (16).

If one excludes classifications where microbes of other genera have been included into the genus *Fusobacterium*, one will nevertheless find that the types of different authors do not harmonize with each other. Some authors (*Varney*, *Slanetz* and *Rettger*) have tried to base their typing upon serological grounds and have obtained distinct serological types, very probably because they have mixed *Leptotrichia* strains with strains of *Fusobacterium*. And even where the authors have dealt with *Fusobacterium* it seems apparent that

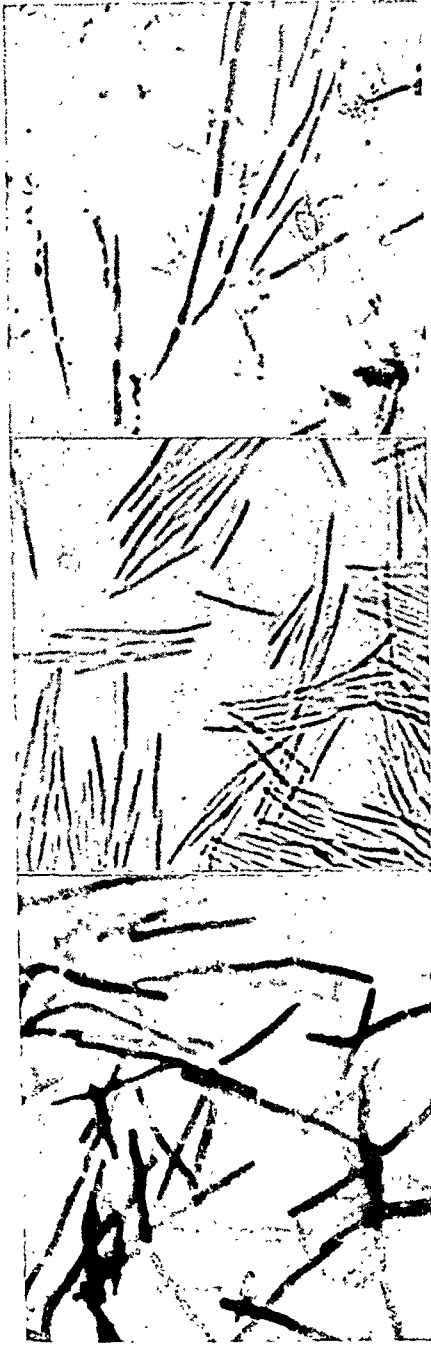


Fig. 1.
Leptotrichia. 48-hour growth on rabbit blood agar. Gram stain. $\times 1000$.

Fig. 2.
Leptotrichia. Unstained. $\times 800$.

Fig. 3.

Leptotrichia in scrapings from teeth. $\times 900$.

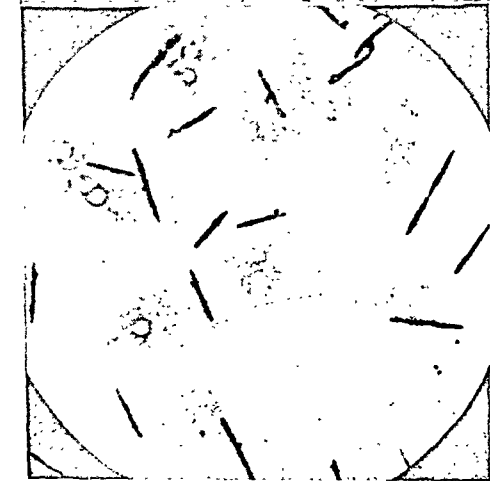


Fig. 4.
Fusobacterium. 24 hour culture in potato medium. Phenol fuchsin. $\times 1200$.

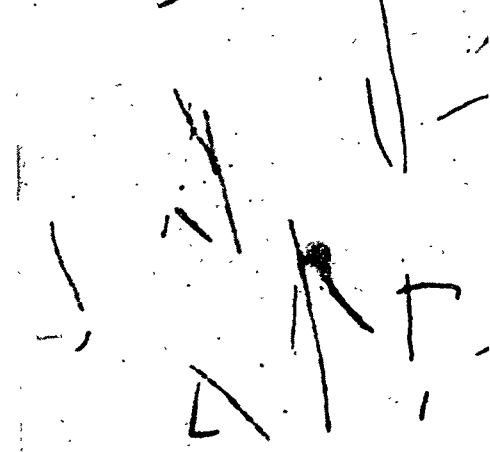


Fig. 5.
Fusobacterium. From «rough» colony. $\times 1200$.



Fig. 6.
Fusobacterium. Flagella staining. $\times 1300$.

even one strain of the same very variable microbe has been divided into different species.

During our work with *Fusobacterium* we were very soon convinced that typing of this microbe on the basis of differences in the capacity for growth of different strains is impossible. W. Bachmann and H. Gregor (17) divided their strains in »Gross- und schnellwuchsig« and in »Klein- und langsamwuchsig«. Such a distinction is not a real distinction, because the first generations very often show differences between individual strains that disappear completely in subsequent generations on artificial media.

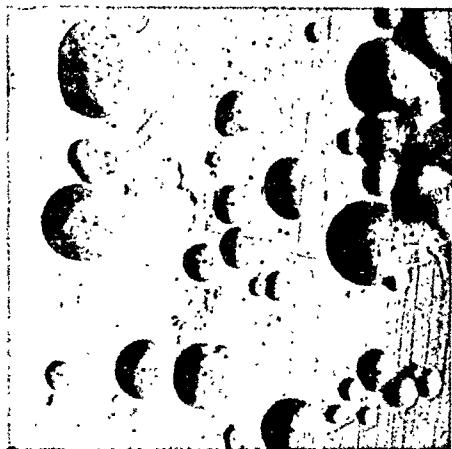


Fig. 7.

Fusobacterium. Large and small *Leptotrichia*. 48-hour culture on colonies on potato extract agar. brain-heart glucose agar. $\times 48$. Oblique penetrating light. $\times 10$.



Fig. 8.

A division into types or species on pure morphological grounds in microbes with such a large variability as that of *Fusobacterium* must a priori be considered illogical. And such differences as may be found in the first generations may disappear later on and the »types« will then appear as identical microbes. We tried at first to divide our 30 strains into 3 types, but this division had to be given up, as the differences turned out to be too inconstant to serve as the basis for a division.

The spontaneous motility has also been used for a division into types (David T. Smith and A. R. Prévot). It is possible that motile strains of *Fusobacterium* may be found, although we did not find any among our 30 strains. All our strains were clearly non-motile, but even these showed flagella.

The biochemical reactions of our 30 strains were clear-cut and constant. And they were identical in all strains, so that they could not be used as a means of division of the strains into biochemical types, while they were well suited to separate *Fusobacterium* from

related microbes, and quite especially from *Leptotrichia*. The *Fusobacterium* is a very constant and strong producer of indol and produces H_2S in somewhat variable amounts. It is a weak fermenter of carbohydrates and gives a terminal p_H in dextrose broth of 6 or above 6. It does not liquefy gelatin or produce gas.

Through these biochemical reactions *Fusobacterium* is clearly separated from *Leptotrichia*, which never produces indol, but is a very strong fermenter of carbohydrates.

»Indol-negative and strongly saccharolytic« strains of *Fusobac-*



Fig. 9.



Fig. 10.

Fusobacterium. »Rough« colonies on *Leptotrichia*. Characteristic growth on ascitic fluid agar, 72-hour culture. on brain-heart glucose agar. $\times 30$.
 $\times 40$.

terium as described by other authors obviously belong to *Leptotrichia* and should be excluded from *Fusobacterium*.

Spaulding and *Rettger* have discussed the possibility of placing their two groups of *Fusobacterium* into different genera. When they placed them into the same genus, however, the reason was that they found strains which seemed to form transitory types. We have not found such strains in our material. If they should be found this fact would only make it still more reasonable that the *Leptotrichia* and the *Fusobacterium* are genera very much related to each other.

Serologically we found that our strains were very closely related, as the absorption of antibodies between strains and sera produced with the different strains of *Fusobacterium* turned out as that between serological types of only insignificant difference. It was impossible to divide the strains into clear-cut serological types. The serological division between *Fusobacterium* and *Leptotrichia* however was very clear and constant, and our results thus are in close agreement with those of *Spaulding* and *Rettger*, who divided their strains

into two serological types. Their type II consists namely of those strains that we feel should be classed as *Leptotrichia* and not as *Fusobacterium*.

After a carefull study of the works of other authors in this field and in accordance with our own studies in our laboratories, we feel that the different types of *Fusobacterium* of the authors cannot be considered correct. It seems just to claim only one species of *Fusobacterium*, *Fusobacterium plauti-vincenti*. If new species should be found these must be studied closely and their characters as *Fusobacterium* stated on solid ground, and especially must the biochemical characters be studied very closely. The large variability of these microbes must be duly considered and it is very probable that an eventual distinction between species must be the result of an analysis of antigens.

III. The systematic position of *Fusobacterium* and *Leptotrichia*.

From what we have said about the characters of the two microbes in question it seems evident that *Fusobacterium* and *Leptotrichia* should be placed in genera closely related to each other. The very fact that these microbes so often have been confused shows that we have to deal with microbes resembling one another and having not been clearly differentiated in our bacteriological systems.

A. R. Prévot (16) in 1938 as the first proposed to place *Fusobacterium* and *Leptotrichia* in two genera closely related in a new family *Spherophoraceae*, in which he also places *Spherophorus necrophorus* (*Actinomyces necrophorus*, i. e. the *Necrosis bacillus* of Bang). In Prévot's system the *Fusobacterium* and *Leptotrichia* are named *Fusiformis*, *Fusocillus* and *Pseudoleptothrix*.

We agree with *Prevot* in his proposition of placing the two organisms in two related genera. But we do not agree to his family of *Spherophoraceae*. This name should namely be characteristic of a family, in which the genera were made up of microbes that formed spheroid bodies in cultures. And this is characteristic of neither the *Fusobacteria* nor the *Leptotrichia*, in which no spheroid bodies occur.

In their *Determinative Bacteriology*, *Bergey et al.* on the other hand, place *Fusobacterium* Knorr in their 9th genus of the 11th family *Bacteriaceae* Cohn. This seems to us to be a logical place for this microbe. *Leptotrichia*, however, is placed among the *Actinomycetaceae* by the same authors. And this is illogical, because the *Leptotrichia* shows none of the characters of the *Actinomycetaceae*. Thus it is not a branching microbe, and it is never seen to form coccoid bodies, but always grows in straight or slightly curved rods. The right place for this microbe must be among the *Bacteriaceae* and not among the *Actinomycetaceae*. We will consequently propose that

Leptotrichia be placed in the 11th family of *Bergey's* system and as the 10th genus of this family. In genus 9 *Fusobacterium* only one species should be distinguished *Fusobacterium plauti-vincenti*, and only one species should be placed in the genus *Leptotrichia*, namely *Leptotrichia buccalis* Trevisan.

The family *Bacteriaceae* is at present a complicated family where many microbes of different characters are placed together. It is probable that this family in the future may be divided into several new families. If so be done, the two genera *Fusobacterium* and *Leptotrichia* should be placed together in a family consisting of rather large rods growing anaerobically or micro-aerophilically. It seems justified to assume that the genus *Bacteroides* in the family *Bacteriaceae* is quite complicated and should be given a thorough systematic study. It also seems obvious that the *necrosis bacillus* of Bang is quite closely related to the *Fusobacterium*. Also on this point further study is necessary.

Conclusions.

1. After a thorough study of the literature dealing with *Fusobacterium* and *Leptotrichia* we find that these microbes are often faulty described and often confused with each other.

2. Based on a thorough morphological, biochemical and serological study of 30 strains of *Fusobacterium* and of 20 strains of *Leptotrichia* we feel justified in stating the characters of the said microbes and in proposing as the logical systematic position of these microbes the 9th and the 10th genus of the family *Bacteriaceae* for the *Fusobacterium* and the *Leptotrichia* respectively.

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A SPORULATING AEROBIC BACILLUS IMITATING CORYNEBACTERIUM DIPHTHERIAE

By Kristian Ødegaard, M. D.

(Received for publication May 10th, 1943.)

In the routine examination of cultures from individuals suspect of diphtheria only members of the *Corynebacterium* will ordinarily be confused with the real *Corynebacterium diphtheriae*, and especially by persons who lack training in this examination.

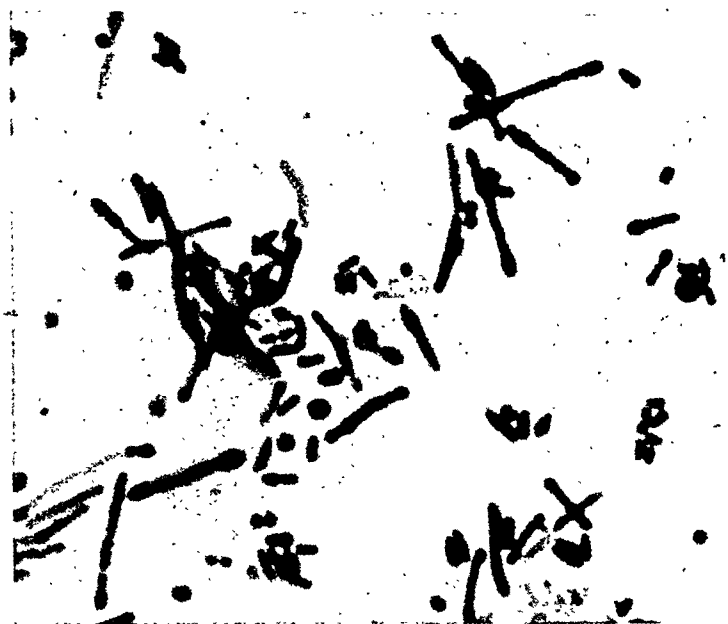
Dealing with some cultures from diphtheriasuspects we found, however, in our institute, a microbe, that resembled the genuine *Corynebacterium diphtheriae* to such a degree, that even trained examiners mistook it for the latter. As we cannot find that this microbe has been described, we take the opportunity to mention it, as it might be that other investigators also may meet it in their cultures.

The strain in question was cultivated from the nose of a boy of 1 year, suffering from an asthmatic condition and suspected of diphtheria. The cultivation was made in the ordinary tube of Loeffler serum. On examination of a smear from the culture after growth for 24 hours, we found that the smear mostly consisted of rods very similar to the typical *C. diphtheriae*. Practically all the rods showed acid-fast granules in the ends, and some also showed such granules in the middle of their body. In the latter case the microbes were somewhat longer than the others. The microbes had a position to each other that completely simulated the appearance of real *C. diphtheriae* in cultures. There were typical V-formations, and many rods crossing each other (see microphoto). When we decided that this microbe was not to be taken as a *C. diphtheriae* without further examination, we had only the length of the microbe to build our doubt upon. For the microbe in question gave the impression of being about twice as long as the real *C. diphtheriae*. On examination of the same culture in living condition it was found that our microbe was lively motile.

Then, of course, it was clear that it could not belong to the genus *Corynebacterium*.

On the following examination of pure cultures this microbe showed the following characters:

The size of our microbe was $3-9 \times 1$ microns, and some specimens had a length of up to 12 microns. It was motile and showed peritrichous flagellas. The rods themselves were weakly Gram-positive, while the granules were strongly Gram-stained, when tested in a cul-



Primary culture. Neisser stain. $\times 1000$.

ture of 20—24 hours' growth. Stained after the Neisser method the granules were typically acid-fast, but they did not show so well in preparations stained with the Loeffler stain. The granules were mostly round and their diameter was slightly larger than that of the rods themselves. The granules situated in the body of the rod were as a rule slightly ovoid with their longer diameter in the direction of the axis of the individual rod.

This morphological picture, however, is changed when the microbe is examined in cultures more than 24 hours old. The granules gradually lose their central staining and develop into typical bacterial spores. The older the microbe grows, the more often one finds these spores only in one end of the rod. In 8-day cultures one will thus find no granules, but typical spores, placed terminally or subterminally, round or ovoid with their longer diameter in the axis of the rod. The diameter of the spores is now, as were the granules, slightly wider than the diameter of the rod. The microbe will thus gain an

appearance that is very much like that of the *Clostridium tetani*. Also free spores are found in such old cultures.

Our microbe grows well on all ordinary media, and its growth is absolutely aerobic. It does not grow at room temperature or at a temperature of 45° C. The colonies are round, smooth and slightly raised, often showing a slight central elevation. The color is greyish and the colony is not translucent. The consistency is viscid, and the colonies are easily taken off the surface of the medium. There is no hemolysis on blood agar, and the growth in broth shows a slight sediment and a diffuse clouding. No pellicle is formed. When shaken the culture shows a silken lustre. On agar plates containing only 1 % agar the microbe spreads over the surface like a spreading *Proteus*.

Biochemically our microbe is a very weak one, that does not ferment any carbohydrate, does not liquefy gelatin, nor produce indole, or coagulate milk or produce H₂S. Neither does it reduce nitrates, but produces NH₃.

No virulence for mice or guinea pigs could be detected, as 0.5 and 1 cc. of a 24-hour broth culture did not cause any illness in the animals.

Several new cultures from the patient were made, but the microbe could not again be cultivated from him. It is thus probable that it may have occurred as a contamination on the Loeffler blood serum, or as a temporary contamination of the mucous membrane of our patient.

Discussion.

The microbe grown from our patient must accordingly be classified as a microbe belonging to the sporulating family of *Bacillaceae* and further as a member of the first genus of this family, *Bacillus*. The granules imitating the acid-fast granules of the *Corynebacteria* must be considered as the primary development of the spores. It is possible to follow this development from granules to the typical spores. When the outgrown microbes show only one spore, while the young forms have mostly two granules, this may be caused by the division of the microbes, or possibly by the reduction of one granule while the other one develops into a spore.

In the *Determinative Bacteriology of Bergey* and coworkers no microbe can be found with characters like our microbe.

On account of the close similarity of our microbe with that of *Corynebacterium diphtheriae*, and on account of its size, we propose the name of *BACILLUS PSEUDODIPHThERICUS MAGNUS*.

Conclusions:

1. An hitherto unknown microbe from the nose of a child suspect of diphtheria is described.
2. The microbe showed a very strong resemblance to the *Corynebacterium diphtheriae* in quite young cultures and might easily have been considered such a bacterium, if the examination had been carried out in a hurry and without due suspicion.
3. This microbe turned out to be an aerobic sporulating bacillus.
4. We propose the name of *Bacillus pseudodiphthericus magnus* for the microbe described.

FURTHER STUDIES ON THE IMPORTANCE OF PLASMA CELLS IN THE FORMATION OF GLOBULIN

(Protein and Plasma Cell Content in Nasal Polypi).*)

By *H. C. Andersen & Jens Bing.*

(Received for publication June 4th 1943).

A number of investigations have been published in recent years showing that there are reasons for assuming that plasma cells and other reticulo-endothelial cells play some part in the formation of globulin. It was shown e. g. by *Bing & Plum* (5) that a feature common to the various affections accompanied by hyperglobulinaemia is an accumulation of plasma cells and other reticulo-endothelial cells in the bone marrow or other places in the organism. The part played by the plasma cells in the formation of globulin has since been underlined by many authors, *Gsell* (10), *Groth* (9), *Jersild* (12), *Markoff* (13), *Rohr* (14), *Wuhrmann & Leuthardt* (16), *Bing* (3), *Apitz* (1), *Gormsen* (8) and *Fleischhacker* (7), who also support their theories on the simultaneous occurrence of plasma cell accumulation and hyperglobulinaemia in various diseases. The connection has also been demonstrated in animal experiments, *Bjorneboe & Gormsen* (6) having found both considerable hyperglobulinaemia and plasma-cell accumulation in rabbits immunized with polyvalent pneumococcus vaccine.

Besides the tissues that are rich in plasma cells, the blood passes so many other tissues from which the globulin might come that it is of interest to investigate the globulin content in closed systems, the protein there either having been formed on the spot or filtered in from the blood.

If it has filtered in, we must assume that the quantity of large-molecular globulins passing into the space will never relatively be more than the smaller-molecular albumins. This will be seen i. a.

*) Investigation made with support (to J. B.) from the P. A. Brandt Fund.

Table 4.

Number	Total-Protein	Albumin	Globulin	Rel. Glob. %	Protein content in the polypus in per cent. of the content in serum
311					
Polypus	6.15	2.93	3.22	52	85
Serum	7.21	4.50	2.71	38	
315					
Polypus	4.52	2.91	1.61	36	60
Serum	7.53	4.66	2.87	38	
316					
Polypus	5.53	3.19	2.34	42	82
Serum	6.71	4.21	2.50	37	
317					
Polypus	5.75				74
Serum	7.77				
319					
Polypus	4.44				55
Serum	8.06				
320					
Polypus	5.95	3.50	2.45	41	73
Serum	8.07	4.95	3.12	39	
326					
Polypus	4.78				67
Serum	7.12	4.36	2.76	39	
327					
Polypus	5.74				80
Serum	7.16				
329					
Polypus	6.14				86
Serum	7.16				
330					
Polypus	7.38	4.84	2.54	34	100
Serum	7.36	4.52	2.84	39	
330 (2/6)					
Polypus	5.84	3.22	2.62	45	96
Serum	6.09	3.64	2.45	42	
331					
Polypus	5.69	3.27	2.42	42	86
Serum	6.63	4.31	2.32	35	
332					
Polypus	5.16	3.02	2.14	41	71
Serum	7.29	4.53	2.76	38	

Number	Total-Protein	Albumin	Globulin	Rel. Glob. %	Protein content in the polypus in per cent. of the content in serum
334					
Polypus	3.53	1.87	1.66	47	53
Serum	6.63	4.24	2.39	36	
335					
Polypus	6.10	4.14	1.96	32	87
Serum	7.05	4.88	2.17	31	
336					
Polypus	4.25	2.94	1.31	31	65
Serum	6.55	4.66	1.89	29	
246I					
Polypus	6.84	3.23	3.61	53	92
Serum	7.45	4.50	2.94	40	
246II					
Polypus	6.11	3.32	2.79	46	81
Serum	7.54	4.50	2.04	40	

from the filtration of proteins through injured glomeruli, as one never finds relatively more globulin in urine than in serum (*Bing* (2)). A closed cavity containing fluid with a varying protein content exists in the spinal canal, and in an examination of the various affections with abnormal protein content in the spinal fluid *Bing & Neel* (4) have shown that it is a common feature of the diseases that cause a relatively globulin increase in the spinal fluid that they are accompanied by an accumulation of plasma cells in the central nervous system and its sheaths.

Nasal polypi being rich in plasma cells, we have gone into the question of whether signs might be found there of these cells being globulin-formers. For this purpose we have, while examining the polypi histologically, made comparative analyses of the protein content in polypus content and serum, employing *Henriques & Klausen's* (11) method of protein determination. In order to procure the polypus content we cut the polypi into pieces, crushed them and, after centrifuging, removed the fluid from the sediment. Simultaneously with the taking of samples for biochemical examination we made slides for histological purposes. To our knowledge there has been no previous investigation of the protein content in nasal polypi.

Eighteen analyses were made from sixteen patients. In 13 cases only was it possible to fractionate the proteins of the polypus fluid; in one case (326) it could not be done on account of lipaemia, in the others (317, 319, 327, 329) there was not material enough for analysis. The results are shown in Table 1, where details are given of the total protein, albumin and globulin content in the polypi and

serum (shown in percentage), of the relative globulin percentage, i. e. the percentage of globulin to the total protein, and of the percentage of polypus total protein to serum total protein.

It appears from the table that the protein content most often, but not always, is less in the polypi than in serum, the content in the polypi being from 53 to 100 per cent. of the content in serum. Consequently, if one would examine whether all the globulin content

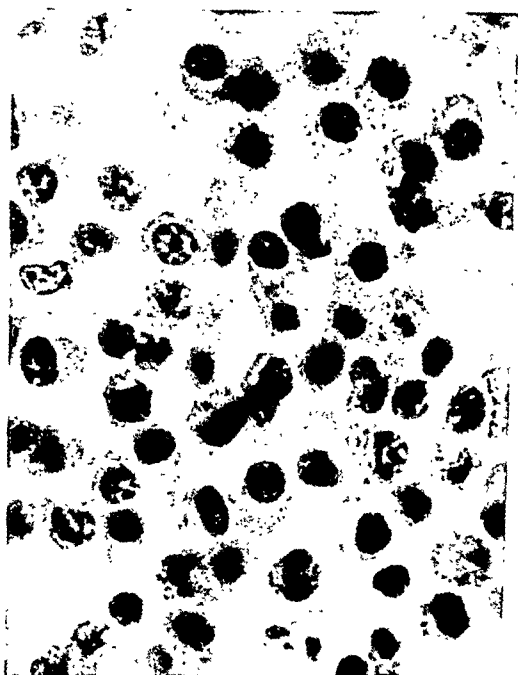


Fig. 1.

Plasma cells from polypus 246, containing 3.6 per cent. globulin, whereas serum from the same patient contained 2.9 per cent.

of the polypus fluid can have been filtered into it from the blood, it is not the absolute values but the relative globulin percentages in polypus fluid and serum that must be compared. It will be seen that the relative globulin percentage is practically identical in 8 cases (315, 316, 320, 330 (2/6) 332, 335, 336), the difference here being scarcely in excess of the limit of error. In one case (331) there is a moderate difference and in three (211, 334 and 246) a distinct difference, there being a considerably higher relative globulin percentage in polypus content than in serum. In two of these cases (211 and 246) the absolute globulin percentage is also distinctly higher in the polypus fluid than in serum.

Histological examination of the polypi whose contents were examined by fractionated protein analysis revealed a number of plasma cells in every case. Their quantity was mostly moderate, but in three

cases there was a very considerable accumulation of plasma cells. One of these (330) was peculiar in that the stainability of the protoplasm varied a good deal, most of them having very faintly stained protoplasm, whereas a minority had the usual characteristic basophili. In the other two cases (211 and 246) the plasma cell accumulation was pronounced, there being both streaks and accumulations of plasma cells besides the diffuse occurrence throughout the polypus (fig. 1). In the second polypus studied from patient 246 both plasma cell accumulation and globulin increase were less pronounced. In addition to the plasma cells there were in all slides numerous polymorphonuclear leucocytes, in most cases mainly eosinophil, and a much smaller number of mononuclear cells.

On making a comparison between the results of the biochemical and histological examinations — carried out independently of each other — we observe the striking fact that these cases (211 and 246) where the globulin content of the polypus is relatively highest — and also actually higher than in serum — are identical with the cases which histologically are outstanding by their great content of plasma cells. Next, it is seen that in a third case (330) with especially many plasma cells the majority of which are but little basophil, there is no definite difference between the proportions of albumin and globulin in polypus and serum. And finally, in the other cases where the quantity of plasma cells was judged to be fairly uniform, there was mostly no difference between the protein fractions in polypus and serum, whereas in two cases (331 and 334) there was relatively most globulin in the polypi.

Thus the investigation showed that in nasal polypi containing plasma cells there may be a formation of globulin, so that it provides further support for the theory that the globulins are formed in plasma cells and other reticulo-endothelial cells. In the majority of cases no difference was observed between the protein fractions of polypus-content and serum, but this may be due either to the circumstance that no globulin had been formed in the polypi in these cases, or that its formation had proceeded so slowly that a balance was set up between polypus content and serum.

It is curious that the proportions between the protein fractions in polypus fluid and serum are identical in the majority of cases, despite the fact that the total protein content in the polypi is mostly less than in serum. However, we find a parallel to this in *Salvesen & Linder's* (15) investigations on the proteins in serum and ascites or pleural fluid, for they too found uniform albumin-globulin proportions, although there was considerably less total protein in the content of the serous cavities than in serum. Similar conditions were found by *Bing* (2) in the course of studies on proteinuria, for in one case (No. 24) the same proportions were found between the protein fractions in urine and serum, although the total protein excretion

was much lower (about one-tenth) than it would have been if the protein content of the glomerulus filtrate had been identical with that of serum. Probably the best explanation in all cases is that only some of the capillaries are permeable to protein, whereas others are impermeable and allow only water and crystalloids to pass.

Summary.

By means of simultaneous protein determinations in serum and in the content of nasal polypi containing plasma cells it is found that the total protein quantity in the polypi represents from 53 to 100 per cent. of the protein content in serum. In the majority of cases the albumin-globulin proportions are identical in polypus and serum, but in some cases relatively more globulin was found in polypus fluid than in serum. This observation may be taken as a support for the theory that the globulins are formed in plasma cells and other reticulo-endothelial cells.

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HEMOCHROMATOSIS.

By *J. H. Vogt.*

(Received for publication June 23th, 1943).

This disease, of which I am going to give a report of 3 cases, was first described by French authors (*diabète broncé*). It was named hemochromatosis by v. Recklinghausen (1889). This name presumes that it is a question of a disease of the blood, but this we now know is not probable.

Sheldon compiled 345 cases till 1935, of which he considers 311 as proved cases. After 1935, the publication of 32 cases from the Mayo Clinic (Butt and Wilder, 1938) must be specially mentioned.

From Norway, Hatlehol described one case in 1927, and gave an account of one case in The Medical Society in Oslo in 1935. On that occasion Olav Hanssen mentioned the observation of two cases. Harbitz published one case in 1930, and Knutsen recently two cases. In the Annual Reports from the Pathologic-Anatomical Institute of the Rikshospital in Oslo from the years 1923 to 1937, I have found 3 cases out of 3518 autopsies, one of which has not been mentioned previously. This indicates that the disease is of about the same frequency in Norway as in the Anglo-Saxon countries.

The present publication of 3 cases, all of which have passed through our medical department during 1941, depends certainly on a coincidence. Sheldon, however, makes a note on the sporadic accumulation of cases in certain regions during limited periods.

Labbé, Boulin and Uhry say in 1934: »C'est cette méconnaissance des formes frustes les plus fréquentes qui explique que l'on porte rarement de ce diagnostic dans certains pays, et que dans certains pays, comme l'Amérique on considère cette maladie comme rarissime.« This »rather caustic remark« resulted in the publication of the 32 cases from the Mayo Clinic.

The most striking feature in the pathologic-anatomical examination is the pigment contents of various organs. There are two pigments, hemosiderin and hemofuscin, and unfortunately both names presume

a hematogenic origin. Hemosiderin, which contains great quantities of iron (Cook 1929), is most richly deposited. It is demonstrable in the pathologic-anatomical preparation after the method of Pearl, or better after the method of Nishimura (1910). It does not dissolve in alkalis, and this may be useful in the chemical determination as done in one of the cases to be presented here.

Hemofuscin is most likely a melanin. It may be the only pigment demonstrable in the skin.

The etiology and pathogenesis of the disease are unknown. In the discussion, the mechanism of the pigment deposits plays a predominant rôle, especially the hemosiderin deposits. Another point of importance is that the usually widespread sclerosis of the organs may be limited to a cirrhosis of the liver. Cirrhosis of the liver is found in 100 % of the reported cases. A similar hemosiderin deposit may be seen in porphyria (Waldenström, 1934), malaria, pernicious and hemolytic anemia, and may be found in animals or provoked experimentally (see Sheldon). These observations, however, have only in a slight degree contributed to the explanation of hemochromatosis. The disease is most certainly not of hemolytic origin. The copper intoxication theory of Mallory is abandoned, except by Creed (1935).

The enormous iron contents of various organs in hemochromatosis obviously must depend on a positive iron balance. Sheldon, however, gives evidence that this positive balance is so slight that the deposit of 40 grammes of iron, as found on the average in hemochromatosis, must take some 40 to 50 years to accumulate. This indicates that the disease may be congenital or may begin to develop in early childhood.

Sheldon also stresses that the cirrhosis *per se* cannot be the cause of the enormous deposits of iron-containing pigments in hemochromatosis.

Alcohol does not play a greater part in the etiology of this disease than in that of the ordinary cirrhosis of the liver.

As to the clinical features and the diagnostic difficulties, this will be sufficiently elucidated by the report of our three cases.

Case I. Working man, born in 1878. A niece has diabetes. He denied abuse of alcoholics, and he has had no venereal disease. He was a slim and asthenic man.

Not having suffered from any serious disease previously, he was suddenly taken by lassitude and general weakness on a warm summerday in 1941. He was admitted to our hospital because of the glycosuria then discovered. He had not had any of the common diabetic symptoms. During the stay in the hospital, he gained 4 kg. in weight on a liberal diet and 28 international units of Retardinsulin in the morning. The blood sugar varied between 250 and 160 mg %, and a slight glycosuria persisted.

His face gave the impression of being sunburned, but this did not cause any suspicion, it being in the middle of the summer.

The skin was described as dry and desquamating. The liver and spleen were not found enlarged.

Only a month afterwards, he was admitted again because of a rapidly developing ascites and crural edema, which had started right after he left the hospital. There was no sign of cardiac failure. The skin of the hand, face and throat was now coloured deeply grayish brown, not desquamating or thickened.

The rest of the body, and especially the skin of the legs, was dirty brown in colour. There were no pigmentations of the mucous membranes. The hairs in the axillae were almost absent, and the pubes very scanty. He was not questioned as to libido sexualis.

After drainage of the ascites, the liver and spleen were still not palpable.

After 10 days in the hospital, the patient died of an intercurrent bronchopneumonia.

Special examinations: Electrocardiography: Isoelectric T_1 , low diphasic T_2 and a little lowered ST_1 and ST_2 . Blood pressure 160/120. The ordinary blood examination showed normal conditions. Takata-reaction negative. Microscopy of feces: Somewhat increased fat content and a considerable amount of poorly digested musclefibres. Feces from three days: 16.8 g. fat and 4.3 nitrogen.

Schlesinger's reaction positive, but negative after two ether-extractions. The urine became darker when boiled with hydrochloric acid, and entirely black when mixed with iron chloride; but there was no sediment. There has possibly been a slight melanin excretion.

The autopsy was performed by Dr. J. Cammermeyer, the Pathologic-anatomical Institute of the Rikshospital in Oslo. It confirmed the diagnosis.

The following is quoted from the protocol: The anterior lobe of the *hypophysis*: Numerous pigmented epithelial cells. *Thyroid*: 27 g., usual consistency, slightly yellowish cut-surface. Microscopy: The alveolepithelium contains considerable amounts of brownish black pigment. In some places all the cells contain this pigment. *Testes*: Weight together: 40 g., firm, elastic. Microscopy: Moderate atrophy with some increase in connective tissue. Here and there pigmented epithelial cells. *Heart*: 330 grammes. On the front side of the right ventricle, there is a moderate thickening of the pericardium in a space of about the size of an American fivecent piece. The myocardium is brownish-pink in colour. Microscopy: Abundant greenish-yellow pigment in the muscle cells. Here and there in the connective tissue some pigmented macrophages are seen. *Peritoneum*: 1500 c. c. of fluid. Numerous black spots in the form of points or dots, as well as in the shape of stripes, especially in the mesentery. Over the coecum, they form a large black surface. *The lymph-nodes* are soft, the size of a pea and faintly pink in colour, but some are considerably darker. *Spleen*: Enlarged (280 g.). Slightly wrinkled, bluish coloured capsule with several yellowish-brown enlargements, up to the size of a coffee-bean, rising a little above the surface. The cut-surface is dark and comparatively firm. Microscopy: In the capsule-enlargements, as well as in the capsule generally and in the trabeculae, there are numerous fine-grained pigments. *Liver*: 1500, very firm with a finely granulated uneven surface. The surface is of a deep black colour on the front side and brownish coloured on the cut-surface. Microscopy: Considerable deposits of pigment in the cells of the liver which in some places show signs of necrosis. The interlobar connective tissue is considerably increased. Also here deposits of pigment are seen, especially in the fibroblasts, and in some bile-ducts too. *Pancreas*: 90 g.; usual lobulation with black pigmentation of nearly half of the surface.

Brownish red cut-surface; comparatively soft consistency. Microscopy: Considerable deposits of black pigment; no Langerhans' islands can be made out; fibrosis. *Suprarenals*: Weight together 15 g. No macroscopic abnormality. Microscopy: In zona glomerulosa clusters of cells are seen to contain fine-grained brownish black pigment. *Kidneys*: Weight together 260. The surface is a little rough, and the capsule is loosened with some difficulty. Microscopy: A few hyaline glomeruli, and small scattered fibrous spots; here and there the tubular cells show some greenish-black pigment. *Bone-marrow*: Some pigmented cells. *Skin from the leg*: In the reticular layer of the corium a few pigmented macrophages are seen round the small arteries.

Iron reaction (Turnbull's blue) in sections from lymph-nodes, pancreas, suprarenals, spleen (capsule) and bone-marrow show partly considerable hemosiderin deposits; only slight deposits in the testes.

Some organs were also examined chemically by Arvid Harboe, stud. med., in the Physiological Institute of the Oslo University.

Iron contents per 100 g. of wet substance: Kidneys 0.3 g., pancreas 0.2 g., spleen 0.7 g., liver 0.7 g.

Case II. Stone polisher, born in 1893. His use of alcohol was moderate. In 1937 he lost energy, became weak in the legs and developed polyuria and polydipsia, while at the same time he lost in weight. On admission to the hospital, he was found to be a little under normal weight, but during his stay in the hospital he gained 6 kg. He was given a diet rich in carbohydrates and gradually 24 international units of ordinary insulin in the morning and 40 international units retardinsulin in the evening. The blood sugar then decreased from 343 to 128 mg. %, and there persisted a slight glycosuria. His diabetes afterwards remained stationary.

In 1937 already he was found to be suffering from cirrhosis of the liver, which was hard and reached from 5 to 6 cm. below the costal bend. Dulness over the spleen was somewhat large, but the spleen was palpable only temporarily, in February 1941.

In 1937, it also was noted that his complexion was of a peculiar greyish-brown colour. This was not, however, very marked, and it varied possibly somewhat in intensity. Thus it was noted in the journal from his next stay in the hospital, the same year, that this colour was less prominent. On the other hand, it was noted during all his several stays in our hospital that the legs were brown-pigmented. When I saw him the first time in 1941, the forearms were greyish-blue, and quite large areas on the legs were of a completely brownish colour. There was no pigmentation of the mucous membranes.

The body-hairs were scarce; he was not asked about libido sexualis.

Special examinations: X-ray examination of the stomach and duodenum showed normal conditions. During a temporary attack of nephritis, the blood pressure was increased. Before and after this, it was 130/90, after a few days' stay in bed. Wassermann and Meinicke II were constantly negative. The electrocardiogram was normal, and likewise the ordinary examination of the blood. Ewald's test meal gave normal values.

The urine was more thoroughly examined right before he died. As a rule Schlesinger's reaction was slightly positive. Ehrlich's was strongly positive also after repeated ether extraction. Porphyrins could not be detected. After boiling with hydrochloric acid or nitric acid, there appeared a considerable quantity of black precipitate, and addition of iron chloride solution brought forth an abundant amount of bluish-black sediment. Most likely this sediment was melanin, but as it was found in the last sample before his death, this assumption could not be proved.

The patient's last admission to the hospital was due to a hematemesis. Two days after his arrival, he suffered another heavy attack of which he died. Autopsy was not permitted; so a piece of skin was taken from the leg for microscopy. A small piece of the liver was also excised for the same purpose. The liver was hard. The surface was slightly uneven and reddish brown in colour. The omentum presented a dense conglomerate of black specks.

The excised specimens were examined microscopically in the Rikshospital by Dr. Olav Torgersen. *Liver*: The surface was irregularly nodular. The parenchyma was partly considerably atrophic, without pronounced degenerative changes. Considerable amounts of brown pigment were observed. The stroma was markedly increased, fibrous and adherent to the capsule. Also the stroma contained considerable amounts of the same pigment. By means of special stains, a part of this pigment was found to be melanin, while some was hemosiderin. *Skin*: Especially the corium contained considerable amounts of brown pigment, and by means of the Turnbull stain the greater part of this pigment was demonstrated to be hemosiderin.

Diagnosis: Hemochromatosis. This diagnosis was mentioned on a clinical basis already in 1937.

Case III. Merchant born in 1890. This man has been drinking heavily from the age of 25 years old and is also addicted to an excessive use of tobacco.

He states that his father, who died of pneumonia of the age of 80 years, had an extraordinarily dark complexion and that a brother, now 60 years old, likewise is very dark-hued. We had no opportunity to examine the brother. A sister of his father died at the age of 72 years; she had then had diabetes for some years. A brother of his father was extremely fat; he «led a fast life» and died 53 years old.

The family of the patient maintains that his skin has been of a very dark hue ever since he was a little boy, and his wife contends that his complexion has not changed throughout the 30 years she has known him. His family is mostly inclined to think that he is born with the present colour of the skin; but, on the other hand, they have paid surprisingly little attention to this peculiarity. Not until 1935 was the patient's attention called to the remarkable colour of his skin by a doctor. The colour is on the whole of a dirty brownish hue. The face and throat are dark greyish-brown. The palms are more red. The legs show dark brown spots and coherent areas. The scrotum and the penis are almost black. The *linea fusca* appears quite distinctly. There are no pigmentations of the mucous membranes.

In 1935 it was found that he had cirrhosis of the liver. The liver reaches 3—5 cm. below the costal margin. The spleen is not palpable.

During the last four years, the growth of hair has become quite scanty, while before he «was a veritable Esau». He still has to shave daily, however. The pubes have a feminin limitation, but there has been no eunuchoid fat-formation. During these four years, libido sexualis has been lacking entirely, and this has been the patient's principal worry. The testes do not appear atrophic on palpation.

Besides his impotence, the patient has sought medical aid on account of anorexia, morning-vomiting, insomnia, temporary edema of the ankles and also temporary staircase dyspnea.

This patient, then, presents, three of the four cardinal symptoms of hemochromatosis, namely: the colour of the skin, cirrhosis of the liver and lack of libido sexualis. On the other hand, he has never had manifest diabetes.

Glucose tolerance tests (1 gram per kg. of bodyweight) have given the following results.

	Fasting	1/2 h.	1 h.	1 1/2 h.	2 h.	2 1/2 h.	3 h.	
Mg. % 1939	118	246	231	166	125			Blood sugar
	0	0	(+)	trace	0			Urin sugar
1940	131	252	280	163	120	112	100	Blood sugar
	0	0	+	trace	0	0	0	Urin sugar
1941 June	118	211	228	189	148	126	95	Blood sugar
	0	0	trace	trace	trace	0	0	Urin sugar
1941 October	120	174	207	166	120	105	102	Blood sugar
	0	0	0	0	0	0	0	Urin sugar

Some of these values may be suggestive of latent diabetes.

Other examinations: The blood pressure varies between 130/85 and 145/85. Several electrocardiograms are normal. Wassermann is repeatedly negative. The Takata reaction was positive in 1939 and negative later. The ordinary blood examination, Ewald's test meal and the serum colour test, all showed normal conditions. Nothing abnormal has been found in the urine. Microscopy of the feces showed normal conditions once; twice the amount of fat has been estimated to be increased, and once the presence of striated muscle fibers has been noted. In the course of three days, however, only 31 of fat and 3.9 g. of nitrogen were excreted with the feces.

During 1941 several serum iron determinations were performed by Gunnar Abrahamsen, medical student, in the Rikshospital. They showed very high values, namely: 291, 276, 294 and 276 γ %.

In October 1941, a piece of skin was taken from the right leg. The microscopic examination, by Dr. Olav Torgersen in the Pathological Laboratory at the Rikshospital, showed considerable deposits of dark-brown pigment in the basal layer of the stratum mucosum of the epidermis and especially in the corium and subcutis. It was found in the fibroblasts of the connective tissues, partly in the macrophages and often around the sweat glands. Special staining methods disclosed an increase in the melanin content of the basal layer. In some places, a few granules of melanin were seen in the connective tissue. The Turnbull test gave everywhere a very strong positive reaction.

Discussion:

One of the three patients was a habitual drunkard. Two of them give a family history of diabetes. One of the patients (No. 3) had a quite interesting anamnesis, with one case of extreme adipositas in the family, one case of diabetes and two cases of extraordinarily dark colour of the skin. The colour of the skin of this patient may be of congenital origin, or at least it dates from early infancy. This is in favour of Sheldon's theory that hemochromatosis may be a congenital, possibly a hereditary condition.

In concordance with what is stressed in the introduction, the laboratory examinations, not referred to in detail (serum colour, H. v. d. Bérgh, osmotic resistance of the erythrocytes, urobilin content

of the urine, and the reticulocyte count), all give *no* ground for the belief that this disease is caused by an increased blood destruction.

The iron contents of the organs of one of the patients (No. 1) have been ascertained. It is difficult to compare these analyses with those compiled by Sheldon, because the latter, as a rule, are given per 100 g. dried substance. The total amount of iron in the liver of our patient can be estimated at 10.5 g. On the average, about 20 g. are found in cases of hemochromatosis, or 40 times more than normal. The amount of iron in the spleen and in the kidneys of our patient was higher than that which is generally found in hemochromatosis. The iron contents of the pancreas is in our case about the average found in hemochromatosis. Sheldon asserts that the probability that the patient has had diabetes increases with an increasing amount of iron in the pancreas.

Serum iron determinations have been made in one of our patients. They are of considerable interest, although they in themselves do not allow of any conclusion. In the case of a disease of such infrequent occurrence as hemochromatosis much time must pass by before there will be collected enough material in the literature to estimate the value of a single laboratory investigation. At present I know only of Heilmeyer & Plötner's analysis in the case of one patient. It showed 199 γ %. Recently in two cases, Knutsen found values from 197 to 241 γ %. In our case, the serum iron approached 300 γ %. As a matter of fact, one should really expect an increase in serum iron in connection with this disease, but the daily positive balance of iron is so small that it does not in itself explain such a great increase as found in the 4 cases available. Such a large increase can only be taken to mean a secondary redistribution or redeposition of the iron in the organism. Some experimental data indicate that such redepositions really take place. Serum iron determination may prove to be of diagnostic help.

The diagnosis of the disease is based, as shown by the above-mentioned cases, on the four cardinal symptoms: cirrhosis of the liver, the colour of the skin, diabetes, and the reduced or entirely lacking libido sexualis accompanied by a reduction of the secondary sex stigmata.

Of these four symptoms, it is only the *cirrhosis* which is found in 100 per cent of the published cases. The skin pigmentations are found in 84 per cent and diabetes only in 78 per cent of the cases. As a rule the cirrhosis is hypertrophic, and the liver, therefore, easily palpable. It causes hematemesis relatively seldom (6 out of 311 cases, according to Sheldon). It should therefore be noted that hematemesis caused the death of one of our patients.

The *skin pigmentations* constitute the first symptom in about half of the published cases. This is especially marked in the case of our patient No. 3. On the other hand, skin pigmentations may be

entirely absent or at least very moderate, as for instance in our patient No. 2. Where there is no colouring of the skin, the diagnosis becomes difficult, because both diabetes and impotence are by no means infrequent in the case of patients suffering from ordinary cirrhosis. Their skin is also often of a somewhat greyish hue.

Excision of pieces of the skin for biopsy plays an important diagnostic part. It should preferably be taken from places not affected by local hemosiderosis from congestion or traumatic injury. But in practice it is difficult to choose places other than the lower extremities. A negative biopsy does not prove anything.

Diabetes is also very often the first symptom. It may be accompanied by signs of defects in the external secretion of the pancreas. Analyses of the feces in our cases No. 1 and No. 3 gave grounds for a certain suspicion to this effect. Before the time of the insulin, the average length of life in cases of hemochromatosis with diabetes was only one and a half year after the patient was obliged to seek medical aid. Now the prognosis is considerably better. The patients may now live several decades. Our patient No. 1 died after one and a half month, No. 2 lived four years, and No. 3 has been pigmented ever since infancy and has had cirrhosis of the liver surely for at least 7 years, but he has no manifest diabetes.

The diabetes is usually of the ordinary type, but some cases of insulin resistance have been reported. This, however, is far from being the rule, as some authors still maintain. There has been reported one death in coma in spite of the injection 1680 international units of insulin in one day. Also cases of increased sensitiveness to insulin have been reported, and this has been explained as a Houssay-effect on the hypophysis or a Long-effect on the cortex of the suprarenal gland by masses of pigment deposits. Bingel thought that administrations of insulin may provoke ascites — as may seem to have been the case with our patient No. 1 — but such cases are so rare that one cannot base any definite conclusion on them.

According to Joslin, there should be reasons for being especially liberal with the carbohydrate-contents of the diet in cases of hemochromatosis.

The lack of libido sexualis, which is the fourth symptom, may in many cases be important for the diagnosis, and at times, as in the case of our patient No. 3, it may be the most worrying complaint of the patient. As to the differential diagnosis it is, however, of no great importance. Diabetics and habitual drunkards, afflicted with cirrhosis of the liver, are very often impotent. In hemochromatosis, it can go so far that the testes become atrophic and the body assumes eunuchoid features. It is believed that the symptom is connected with pigment deposits in the testes, hypophysis and suprarenals. Testosterone treatment, as far as I know, has never been tried. One of our patients (no. 3) indicated a temporary relief after an injection treatment of

vitamin B. He has also been given 4 injections of 10 mg. »Testoviron« and 3 injections of 25 mg. without effect.

The other conventional clinical methods of examination give but little of interest. The blood pressure is considered low for the age of the patients. This is said to be caused by the influence of hemosiderin on the suprarenal cortex, especially by those who maintain that the colour of the skin also is caused by a reduction in the functions of the suprarenal cortex. As to our patients, the blood pressure was permanently increased in one of them; and one had a temporarily increased blood pressure during an acute nephritis; one patient had normal blood pressure.

Electrocardiography has been performed in 8 previous cases; five of them were found to show pathological changes. The same applies to two of our 3 patients.

The basal metabolism seems unaffected, in spite of the considerable deposits of hemosiderin in the thyroid. This is rather strange, considering that deposits of hemosiderin is thought to be the cause of the reduced functions of the pancreas, hypophysis and suprarenals. Sheldon's material shows most often a slight increase in the basal metabolism. In the Mayo Clinic, on the other hand, a moderate decrease was found more often. Only one of our patients was examined in this way, namely No. 3, who showed a metabolic rate of 127 and 123 per cent.

In the urine, there is nothing of diagnostic interest, as far as an ordinary examination is concerned, save the usual diabetic findings. Melanin has previously been found once (Uhlenbruck). One of our patients appears to have had melaninuria just before his death, but this cannot be stated with certainty. Melanin is ascertained by precipitation with the iron chloride solution used in the Gerhardt-reaction, or by boiling with hydrochloric acid. According to Helman, it can be redissolved in alkalis and again precipitated with hydrochloric acid. From the solution in alkalis according to Kobert, the melanin can also be precipitated with alcohol as a brownish red, sticky mass.

Peyton Rous' report in 1918, wherein he states that it is possible to detect granules of hemosiderin in the centrifugate of the urine, appears to be of considerable diagnostic interest. The method is mentioned with interest in all publications on the subject of hemochromatosis, but the majority of authors have depended more on the biopsy. I have time and again examined the urine of our living patient and have found pigments several times, and after colouring, according to the method of Pearl, blue particles or granules have also appeared. They were, however, remarkably numerous. In tests from 20 patients, who assuredly were not afflicted with hemosiderosis in any form, I found the same blue particles in nearly every case. Most likely the air in the laboratory or the test-tubes have contained ferruginous dust. The method has, therefore, been of no help to us.

In 1939 Fishback suggested a method by which iron can be detected in the skin in vivo and after death, which also is based on Pearl's principle. Fishback gave a small intradermal injection of a mixture of sterile solutions of $n/100$ hydrochloric acid and 0,5 per cent potassium ferrocyanide. The injection is said to produce a stinging pain, lasting for a moment, but is otherwise of no consequence. In cases of hemosiderosis, a blue colour is said to appear immediately, which becomes a deep blue during the first five minutes. In the course of two days, a peripheral blush appears. Both the blue colour and blush disappear after two weeks. On the other hand, in case of a negative reaction, the colour in the centre will be entirely white.

We have tried this method on No. 3 of our patients. An intracutaneous injection was given on the forearm. A faint greenish tint appeared, which disappeared within 15 minutes. On the leg could be seen a more distinct greenish blue colour around the stab, about like when a vein is seen through the skin. But it could not be called »deep-blue«. The colour disappeared after a few hours. On the examiner's forearm appeared only an ordinary white papule. The pain was moderate and lasted only a few seconds. A negative result of the test clearly is of no diagnostic importance.

Summary.

An account is given of three cases of hemochromatosis. The diagnosis was verified by autopsy in two cases and by biopsy of the skin in one case. All three patients were men. Two of them denied the abuse of liquor.

A survey is given of the main points in the discussion of the pathogenesis of the disease and of the symptoms. The particular features of the individual cases were: Patient No. 1 developed ascites and died a few weeks after the diagnosis was made and insulin treatment instituted. Patient No. 2 died after hematemesis. The last urine examined gave a rich black precipitate with ferric chloride and with hot mineral acids (melanin?). Patient No. 3 has no manifest diabetes. His chief complaint is lack of libido sexualis. This patient has apparently been pigmented from infancy, and the family history indicates the possibility of inheritance.

The serum iron of the last patient was determined. It was repeatedly found considerably increased.

The method of Peyton Rous, according to which the finding of hemosiderin in the urine is said to be of diagnostic help, was of no value to the author, the analysis of normal urine also simulating the positive test. This is explained by assuming the presence of ferruginous dust in the laboratory.

In the case of patient No. 3, the test suggested by Fishback was tried. It consists in an intradermal injection of very dilute hydrochloric acid and potassium ferrocyanide. The test gave a positive result, although the colour obtained was slightly blue, not deep-blue as stated by Fishback.

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ACCELERATED DEVELOPMENT OF SPONTANEOUS LEUKEMIA AND MAMMARY CARCINOMA IN MICE AFTER INGESTION OF CARCINOGENIC HYDROCARBON*)

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(Received for publication Sept. 10th 1943).

Previously, tumors produced experimentally in mice — for instance, by painting of the skin or by subcutaneous injection of carcinogenic substances — were taken to result from the carcinogenic influence alone as the outcome of the activity of an agent with capacity for tumor production at the very site of application and nowhere else. Gradually, however, investigators have become more inclined to look upon such experimental tumor production as the final result of several factors, among which the carcinogenic influence is only one factor — although a most essential one. Among the other factors, mention has to be made of the hereditary disposition to the development of the very type of tumor that makes its appearance in a given experiment.

The carcinoma of the skin after painting with carcinogenic hydrocarbon or tar in mice represents one extreme point, where the carcinogenic influence is of predominant significance, while the »spontaneous« tumors in the inbred mouse strains constitute the other extreme point, where the development of a tumor is almost entirely dependent on the hereditary disposition alone. That the painting with tar in the first-mentioned experiment is not the only decisive factor is evident from the differing reaction of different mouse strains to the same application of tar. In some strains the tumor develops rapidly, in others very slowly; in some strains a tumor develops in nearly all the treated animals, in other strains only in a fraction. Indeed, some animals are able to tolerate even protracted application of the carcinogenic substance without reacting with any tumor growth

*) The studies here presented were carried out with the aid of grants from Anders Hasselbalchs Leukæmifond, Kong Christian X's Fond and from the National League for Combating of Cancer.

at all. Correspondingly, it can be demonstrated that also the hereditary disposition in the inbred mouse strain is not the only significant factor in the tumor development, as various influences may accelerate the tumor development and increase the incidence of tumor growth in the strain considerably.

These observations have led to a change in our view of experimental »tumor production« so that the development of tumors no longer is considered dependent on the employed agent alone, but is now looked upon as an acceleration of a tumor disposition which also in the absence of the experimental influence would have had a certain chance of resulting in the development of the kind of tumor concerned.

This view of the tumor production in general as acceleration of an inherent tumor tendency of the tissue concerned is really evident from a great number of experiments reported in the course of years, even though it was not clearly formulated till 1939—40.

Long before that time, however, Clara Lynch (1926—28) had advanced this view concerning tumors of the lungs, but it was generally looked upon as something particular to the lungs until reports appeared simultaneously from U.S.A. (Mider & Morten, 1938—39; Brues & Marble, 1939) and Denmark (Engelbreth-Holm, 1939) on acceleration of the development of leukemia in inbred mice by painting with tar or carcinogenic hydrocarbon. When, shortly after, it was found that the growth of mammary carcinoma in mice likewise can be accelerated by carcinogenic hydrocarbons (Engelbreth-Holm & Lefèvre, 1941), it seemed probable that also the earlier experiments in which tumors were »produced« by Roentgen irradiation (*e. g.*, Furth & Furth, 1936) properly are to be interpreted as an accentuation and acceleration of a development of tumors by the irradiation that would have appeared anyhow, albeit less frequently and later in the life of the animal.

Such an acceleration of tumor development may be demonstrated most easily in inbred mouse strains in which a certain form of tumor makes its appearance in a constant percentage of the animals in a certain age-class. Here it is practicable to carry out comparative studies on influences of most different character and ascertain whether they accelerate or, possibly, inhibit the spontaneous development of tumors.

Carcinogenic hydrocarbons have an accelerating effect on leukemia as well as mammary carcinoma and other forms of tumors both on subcutaneous injection and application to the skin (Engelbreth-Holm & Lefèvre, 1941). But this effect is concealed if a local tumor growth induced at the site of application proves fatal to the animals before the accelerated spontaneous tumor is able to manifest itself.

In the present work an account will be given of experiments undertaken to see whether alimentary application of 9:10-dimethyl-1:2-

benzanthracene also is able to accelerate the development of tumors in one of our mouse strains.

Mice of the Street strain were employed for this purpose. Through several years the breed of this strain has shown a constant spontaneous incidence of leukemia of 1—2 % among all the animals, and mammary carcinoma of 25—30 % among the females. The leukemia makes its appearance at the age of 10—12 months, while the mammary carcinoma is most frequent in pluriparae which are 14—23 months old. The Street strain is not yet pure inbred — *i. e.*, the inbreeding has not been carried through for the 18—20 generations usually required for this designation — but as the tumor incidence is constant, the animal is very suitable for the present experiments.

The experiments were carried out so that every other animal in a number of litters was treated, while the remaining, the sibs of the treated animals, were left untreated as controls. The experiments were carried out as follows: 0.1 cc. of a 0.1 % solution of 9:10-dimethyl-1:2-benzanthracene was given by mouth in each dose through a glass tube introduced in the oesophagus — *i. e.*, 0.1 mg. of the hydrocarbon was ingested with each dose. In a greater part of the experimental period this dose was given once a week, in the remainder, 2—3 times a week.

In two experimental series, which here will be dealt with under one, altogether 175 mice were treated in this way, while 142 untreated sibs served as controls.

A great many of the treated animals died from the treatment, as the repeated introduction of the »stomach tube« not infrequently resulted in a perforation or introduction of the tube into the trachea. Naturally these experimental complications have compromised the experimental results not inconsiderably, as many of the treated animals died before they had any chance to develop the possibly accelerated tumor. Nevertheless, the experiments have shown indisputably that the given treatment accelerates the development of the malignant tumors characteristic of this strain.

Table 1 gives the figures for the treated animals and the controls with regard to development of leukemia. The number of treated animals is given as 68, which is the number of living treated animals at the observation of the first case of leucosis. No less than 107 out of the original 175 animals had died before this juncture. At this point of time 102 controls were still alive.

Table 1.
*Development of Leucosis in Mice after Ingestion of
9:10-dimethyl-1:2benzanthracene.*

Age in months at death of the animal	4	5	6	7	8	9	10	11	12	Total of leucosis	Incidence
Treat group 68 animals	1	2	4		1		1	1		10/68	15 %
Control group 102 animals			1	1	1					3/102	3 %

The character of the leucotic changes is recorded in Table 2.

Table 2.
Leucotic Changes in Treated Animals and Controls.

Mouse No.	Sex	Age	Total dose	Weight	Changes
<i>Treated animals</i>					
20155	M.	4 $\frac{1}{2}$	2.1 mg.	26 g.	Generalized lymphogenous leucosis.
20124	M.	5 $\frac{2}{3}$	2.1 "	22 "	Generalized stem-cell leucosis.
20145	F.	5 $\frac{2}{3}$	2.5 "	23 "	Leucotic hyperplasia of the thymus.
20060	F.	6	2.6 "	25 "	" " " "
20018	M.	6 $\frac{1}{2}$	2.6 "	20 "	Generalized stem-cell leucosis.
20103	F.	6 $\frac{2}{3}$	2.5 "	20 "	Atypical hyperplasia of the thymus.
20007	M.	6 $\frac{1}{3}$	6.1 "	20 "	Leucotic hyperplasia of the thymus.
20320	M.	8	2.3 "	28 "	" " " "
20314	F.	10	3.3 "	22 "	" " " "
20296	F.	11	3.6 "	35 "	" " " "
<i>Control animals.</i>					
20490	F.	6		21 "	Generalized stem-cell leucosis.
20022	M.	7		20 "	" " "
20472	M.	8		32 "	Leucotic hyperplasia of the thymus.

In a great many of the treated animals the disease was manifest only by hyperplasia of the thymus — which was so marked that most often it gave rise to dyspnea — and hydrothorax from compression of the heart and lungs, together with leucotic infiltrations in the pericardium and in the lungs along the bronchi. That the leucotic processes were not more conspicuous is due to the fact that these animals have not reached the full development of the morbid features of the lesion because they died from the treatment or, in some cases, the hyperplasia of the thymus was so marked that the accompanying compression of the heart and lungs proved fatal. As to the leucotic character of the hyperplasia of the thymus there can be no doubt — as a rule it is the earliest leucotic manifestation. In the present material the leucotic cases were distributed evenly on males and females, and the age of the animals varied from 4 to 11 months. There was no pronounced shift in the time for the appearance of this lesion in the treated animals as compared to the general rule, even though the development of leucosis at the age of 3—4 months in untreated animals is rare. That the disease did not appear more frequently in the very young age-classes, corresponding to the acceleration observed now and then on painting with carcinogenic hydrocarbon must simply be due to the fact that for technical reasons it is impracticable to commence feeding the mice carcinogenic hydrocarbon by tube before the animals are full-grown.

That the treatment has brought about an acceleration of the development of leucosis is unquestionable. As is evident from Table 1,

leucosis was about five times more frequent in the treated animals than in the controls.

On comparison of the treated animals and the controls with reference to the development of mammary tumors we find the figures presented in Table 3.

Table 3.
*Development of Mammary Carcinoma in Mice after Ingestion
of 9:10-dimethyl-1:2-benzanthracene.*

Age in months at appearance of tumor	7	8	9	10	11	12	13	14	Total	Incidence of mammary tumor
Treated group 22 animals	1	3	2	2	1				9/22	40 %
Control group 57 animals		1			1	1		1	4/57	7 %

Naturally the number of animals here is much lower than above, because this account covers merely the females, as no mammary tumors have been observed in the males of this strain.

As in the account of the leucosis morbidity, the number of animals here reckoned with refers to those that were living at the appearance of the first mammary tumor among the treated animals.

From Table 3 it is evident that the treatment has brought about a considerable acceleration of the appearance of tumors in these animals too. The incidence of tumors is again five times greater here than in the controls, and the point of time for the appearance of the tumors and for the death of the animals has been advanced considerably. It is to be mentioned, however, that none of these animals died in connection with the introduction of the tube — which would otherwise have given a false »advancement« of the death age.

Among the controls, mammary carcinoma was observed only in 7 % as against the ca. 30 % commonly found for this strain of mice, but this is due to the fact that here the experiment was concluded when the animals were 14 months old, while, as mentioned before, the spontaneous mammary tumors usually do not develop till the animals are 14—23 months old.

Particular data concerning the individual cases are given in Table 4, from which it is evident that the acceleration was manifest not only in the earlier appearance of the tumors and their greater incidence but also in the occurrence of multiple carcinomas, which is relatively infrequent in untreated animals. Further, it is also a striking fact that of the 9 treated mice with malignant tumor of the breast 4 had never had any young. The outcome of this experiment reminds in many respects of our previous experiments on acceleration of mammary carcinoma by means of painting with, or subcutaneous injection

Table 4.

Data concerning Treated Mice and Controls with Mammary Carcinoma.

Mouse No.	Age	Total dose	Weight in. g.	No. of par-turitions	Changes
<i>Treated animals.</i>					
20290	7	1.9 mg.	38	2	Cystic adenocarcinoma.
20055	7 ³ / ₄	2.8 »	26	0	Polymorphocellular sarcoma.
20233	8 ¹ / ₂	2.2 »	45	0	3 adenocarcinomata.
20341	8	2.3 »	20	2	Solid carcinoma.
20173	9	3.5 »	22	1	Adenocarinoma.
20248	9 ¹ / ₃	2.6 »	22	0	Solid carcinoma with pulmonary metastases.
20275	10	3.0 »	40	2	Adenocarcinoma with pulmonary metastases.
20386	10	3.2 »	21	0	Solid carcinoma with pulmonary metastases.
20302	11	3.7 »	28	3	Adenocarcinoma with pulmonary metastases.
<i>Control animals.</i>					
20355	8 ¹ / ₃		32	2	Solid carcinoma with pulmonary metastases.
20463	11		34	2	Solid carcinoma.
20109	14 ¹ / ₃		41	4	» »
20169	14 ² / ₃		32	1	» »

tion of carcinogenic hydrocarbon (Engelbreth-Holm & Lefèvre, Engelbreth-Holm, 1941).

Besides the changes here described — leucosis and mamary carcinomas — a few other changes were observed in the treated animals that have to be ascribed to the treatment. This applies in particular to local changes in the stomach. Nearly all the animals which had been given the carcinogenic hydrocarbon by way of the tube showed hyperplasia of the stratified squamous epithelium of the fundus of the stomach with keratosis, a tendency to papilloma formation which evidently is proportional to the duration of the treatment — just like the papillomas on the skin after painting. In the longest living animal — 13 months after the commencement of the experiment — several papillomas (measuring up to 3—4 mm.) were seen, but manifest carcinoma was not observed in any instance.

Further, several of the animals showed inflammatory changes in the lungs with a granulation tissue rich in fibrin. Presumably the changes are to be interpreted as resulting from chronic lipid pneumonia produced by the oil in which the hydrocarbon was dissolved; for, on repeated administration of this solution through the tube, it cannot be helped that some oil now and then enters the trachea too.

Comments.

By ingestion of 9:10-dimethyl-1:2-benzanthracene in doses of 2—3 mg. distributed over weekly doses of 0.1 mg. it has thus been possible in mice to produce a considerable acceleration of the development of leucosis and mammary carcinoma, as the incidence of both types of tumors in the treated animals was about five times greater than in their untreated sibs. Besides, the development of the lesion was also advanced chronologically — at any rate as far as the mammary tumors are concerned.

Very likely this acceleration is due to absorption of the carcinogenic hydrocarbon from the digestive canal. It is possible, however, that a part of this hydrocarbon is absorbed from the air passages as autopsy often showed changes in the lungs that suggested that oil had entered the air passages. There is no doubt, however, that the greater part of the introduced hydrocarbon passed through the stomach. This is evident from the finding of papillomatous hyperplasia of the mucous membrane of the fundus of the stomach and also from observations made at the autopsy. In every instance the autopsy was performed in ultraviolet light, and here the fundus of the stomach shows a strong fluorescence even many hours after ingestion — owing to inhibition of the mucous membrane with the hydrocarbon.

The fact that ingestion of a carcinogenic substance may accelerate the development of such spontaneous tumors as the organism in some degree is disposed to is of considerable interest. Whether it may be of practical significance is yet impossible to decide. Theoretically it is possible that carcinogenic substances in the food may have influence upon the development of malignant tumors — the more so as Widmark has demonstrated that carcinogenic substances are formed, for instance, in fat and meat on frying. But here, no doubt, the quantity of these substances will differ greatly from that employed in our experiments. Finally, it is impossible a priori to imagine in what degree the tumor development in the individual case may have been influenced by the circumstance that the tumor disposition of the animal is increased through the beginning inbreeding of this mouse strain.

Summary.

Peroral administration of 9:10-dimethyl-1:2-benzanthracene (0.1 mg. 1—2 times a week, altogether 2—3 g.) increases the tumor incidence among Street mice for leucosis from 3 % to 15 %, and for mammary carcinoma from 7 % to 40 %; and it accelerates the tumor development at any rate as far as cancer of the breast is concerned.

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EXPERIMENTAL STUDIES ON THE SIGNIFICANCE OF GLUTAMIC ACID FOR THE GROWTH OF MALIGNANT TUMORS

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(Received for publication Dec. 19th 1943).

Since the observation of *Pasteur*,¹⁾ that a culture of *penicillium glaucum* in a solution of racemic ammonium tartrate will attack the d-component only, the difference in biological action of a ferment on the antipodic forms of an optically active substrate has grown to become a factor of decisive significance for a great number of important chemical processes in the living organism and has been the foundation for the elucidation of biological problems of great importance. Also the hypothesis of F. Kögl²⁾ on the ethiology of cancer is build upon this stereochemical foundation, supported by a series of careful experiments which have already called forth a whole literature within cancer research.

The numerous attempts to an experimental verification of the observations by Kögl, which have been published by several authors,³⁾ have for a part been of analytical-chemical and for a part of enzymatic-biological character. In some cases agreement with Kögl's results has been found in others this is not the case and numerically the latter result for the present is predominating.

But still it is acknowledged by authors belonging to the more prominent class in cancer research that Kögl's hypothesis has not yet been and probably can not be definitely settled, neither confirmed nor rejected, with scientific certainty until our knowledge in the cancer problem especially the problem of the growing of tissues, malign as well as sound, has considerably improved. Here it may suffice to make the following quotations from the literature in 1942.

*Emil Abderhalden*⁴⁾ writes: «Wir betrachten die ganze Forschung über das Vorkommen von Aminosäuren der d-Reihe in Tumorzellen keineswegs als abgeschlossen. Die Feststellungen von Kögl u. Erx-

leben sind derart präzise dass sie zur weiteren Forschungen nötigen«. *Hans v. Euler*⁵⁾ in his book »Biochemie der Tumoren« declares: »Nach unserer Auffassung ist den Gegnern Kögls eine Wiederlegung seines experimentellen Ausgangspunktes der sehr häufigen Anwesenheit von d-Aminosäuren in Tumoreiweiss nicht überzeugend gelungen« and *K. Hinsberg*⁶⁾ in his book »Das Geschwulstproblem« ends his discussion of a greater part of the literature for and against Kögls hypothesis in the following words: »Bei der Frage, ob der Obertitel für Köglsche Arbeiten »Ätiologie der Tumoren« berechtigt ist, ist zu sagen, dass diese Frage heute schwerlich entschieden werden kann, da über das Problem noch zu wenig bekannt ist«. By the now existing stage of science it therefore may seem justified to take Kögls hypothesis in its present form as a preliminary base in the planning of experimental studies on the growth of tumors, hoping that, besides bringing practical results, they may contribute to an elucidation of that much disputed hypothesis.

From a purely chemical point of view the hypothesis of Kögl seems quite plausible. As it is well known its aim is to show, that the proteins of the cancer cells are not built up exclusively from amino acids of the l-configuration, as are the proteins of the normal cells, but contain, at least partially, amino acids and especially glutamic acid of the antipodic d-configuration. The growth of the cancer cells will for this reason not be checked by the controlling factors which otherwise bring about the cessation of growth when the tissue has reached its normal state. These growth controlling factors, for the present imperfectly known, are probably of enzymatic character and of asymmetric structure, specifically built to react only with »natural« proteins i. e. proteins built from l-amino-acids only.

This explains according to Kögl the autonomous infiltrative growth of the cancer cells accompanied by necrosis in the surrounding tissues and certain pathological changes in the metabolism of the organism. As mentioned above the glutamic acid is supposed to have a dominating position among the amino acids, which occur in d-form in cancer tissues. According to Kögls experimental results the malignancy of a tumor seems to be the more pronounced the more d-glutamic acid it contains.

The cause of the abnormal proteinsynthesis which according to Kögl is the base of the malignant growth may perhaps be sought in some spontaneous change in the specificity of certain proteases and probably also in other enzymes of the organism brought forth in a presently unknown way.

The optical specific action of the enzymes was originally assumed to be absolute and unchangeable, but this has appeared not to be the case. Even *Frankland*⁷⁾ has shown that while a fresh culture of bac. aetacet. does not attack \rightarrow Ca-glycerate, cultures which have been applied for some times to decompose racemic Ca-glycerate will

also attack \div Ca-glycerate when the supply of the $+$ form has been consumed. That enzymatic reactions often proceed not in one stereochemically determined direction only (absolute specificity), but also, even to a less degree, in the stereochemically opposite direction (relative specificity) is a well known fact and also that the optical specificity may be altered by influences of physical as well as chemical nature and by changes in the medium. As examples of this may be mentioned the investigations by *Willstätter, Kuhn and Bamann*⁸⁾ on the effect by changing the substrate concentration, the investigations by *H. A. Krebs*⁹⁾ on the effect of drying and grinding the enzyme containing tissues and the action of such substances as cyanide and octylalcohol and the experiments by *Bamann*¹⁰⁾ on the action of alkaloids.

In enzymatic reactions, in spite of the often very complicated and usually not perfectly understood course of reaction, we may assume, at least as a first approximation, that the mass action law is valid in dilute solutions. It is generally assumed that enzyme and substrate in these reactions first unite, forming thereby a labile addition product wherein the substrate is activated and that the main reaction is performed by this additive compound whereupon the enzyme is again split off from the reaction product. As to the two addition compounds of the same enzyme with the antipodic forms of the substrate must be of diastereomeric configurations their decomposition velocities v_+ and v_- will, as a rule, be different. If these velocities are assumed proportional to the concentrations i. e. the decompositions to proceed unimolecularly, we should expect that for the rates of formation of the two stereomeric final reaction products we should have:

$$\frac{v_+}{v_-} = \frac{k_+ \cdot K_+ \cdot [S_+]}{k_- \cdot K_- \cdot [S_-]}$$

where k stands for the velocity konstant of decomposition, K for the affinity constant of the formation of the addition product and $[S]$ for the concentration of the substrate.

By changing the substrate concentration there consequently should be a possibility of bringing forth a shift in the stereochemical composition of the reaction product.

To investigate whether glutamic acid is of importance or not for the growth of tumours, especially in relation to Kögl's hypothesis, the experiments in the present paper were planned. The idea was to produce, if possible, a change governed by the mass action law in the stereochemical result of the pathological protein synthesis which according to Kögl is the base of the malignant tumor growth, in such a manner that the growth of the tumor is stopped in its initial stage and its malignant character reduced.

As for the present it is necessary to leave the matter in abeyance, from where the relatively considerable quantity of $d(\div)$ glutamic acid

comes, which according to Kögl is necessary for a strong growth of tumors, the mode of proceeding in these experiments should mainly consist in producing a considerable accumulation of the natural l(+)glutamic acid in the tissues surrounding the tumor, thereby forming a sort of barrier below and around the malignant tissue whose growth is influenced by one or more enzymes with spontaneously changed specificity, the tumor being at the same time protected, to a certain degree, against access of d(÷-)glutamic acid. A peroral administration of l(+)glutamic acid could not be expected to have the same effect.

The experiments were made with white mice of an inbred strain («Street mice»). Besides a considerable number of preliminary experiments made partly to make certain that the applied substances did not show any toxic effect with the animals partly to develop the experimental technique, of actual experiments the following ones were made.

Experiment A. In this experiment »Crocker sarcome 180« was transplanted to the left side of 44 mice and the animals numbered. After the lapse of 5 days all tumors had taken in a uniform manner; in the mean time 5 had died from intercurrent disease (enteritis). The mice were divided into two groups. Group I comprised 22, group II 17 mice. The mice of Group I were, with intervals of 5 days, given in total 4 subcutaneous injections of 0.1 ml. of a suspension of 2 g. solid l(+)glutamic acid (finely powdered and sifted through sifting cloth number 8) in 10 ml. of a 0.1540 molar solution of NaH—l(+)glutamate containing 2 % gelatine as a stabilizer. At the same times the mice of group II were injected with 0.1 ml. physiological NaCl-solution containing 2 % gelatine. The fourth day after the last injection all the mice were killed by coal-gas and the tumors were dissected out, free from other tissues, by Professor Engelbreth-Holm and measured in three directions at right angles to each other. The tumors from each group were weighed jointly.

In group I the average weight of the tumors was 1.26 g.

In group II „ „ „ „ „ „ „ 1.47 g.

The group treated with l(+)glutamic acid thus shows a reduction of the tumors of 14 % in comparison with the untreated group.

Experiment B. This experiment was carried out in the same manner as experiment A, but the treatment began already 2 days after the transplantation, and the total number of Street-mice with transplanted Crocker sarcome 180 was 40, which were divided into two groups of 20 each. The mice of Group I had, with intervals of 2 days, in total 4 subcutaneous injections of 0.2 ml. of the same suspension as applied in experiment A; i. e. the double of the dosis

given in A. Group II was treated in the same manner as in A, the injections however of the same size as in group I in experiment B.

The mice were killed 2 days after the last injection and in every detail treated as in experiment A. The result was:

In group I the average weight of the tumors was 0.35 g.

In group II , , , , , , , 0.50 g.

In the group treated with l(+)-glutamic acid there was thus in tumor weight a reduction of 30 % in comparison with the untreated group. In experiment A and B the average reduction of tumor weight thus is approximately proportional in the applied dose.

To be able to decide whether or not a specific action of the l(+)-glutamic acid had been acting in the above mentioned experiments another experiment was planned which besides a control series, without any treatment at all, should contain a second control series treated with d, l-glutamic acid.

In the execution of this experiment the d, l-glutamic acid, applied in the same manner and with the same dose as the l(+)-glutamic acid, contradictory to previously made preliminary experiments, however showed a toxic action with the result that out of 20 mice 12 died after the second injection. The dissection showed the skin rounding the injection channel strongly discoloured and brittle. A special experiment was therefore started to test the toxicity. 8 sound mice having no tumors transplanted were injected with the same dose of d, l-glutamic acid (0.2 ml.) which had appeared to be toxic. 5 of these mice died after the third injection. The dissection showed the same symptoms as described above. The experiment was discontinued, and experiment to replace d,l-glutamic acid with l(+)-aspartic acid were started. This acid had no toxic effect when applied in the same dose as the l(+)-glutamic acid. Meantime however a series of simultaneous experiments with diminished doses of d,l-glutamic acid had shown that even a reduction to the half of the toxic dose was sufficient to eliminate every noxious effect, and it was therefore determined to use this result in the planned experiment, the application of d, l-glutamic acid being able to yield certain informations which could not be expected from the application of aspartic acid.

Experiment C. Crocker sarcome 180 was transplanted to 60 Street-mice, which were divided into 3 groups each of 20. The treatment started 2 days after the transplantation. The mice of Group I were given 0.2 ml. of a suspension of 2 g l(+)-glutamic acid (solid) in 20 ml. 0.1540 molar solution of NaH-l(+)-glutamate to which was added 2 % gelatine i. e. practically the same dose as was given in group I of experiment A. The mice of group II were given 0.2 ml. of a suspension of 2 g. d,l-glutamic acid (solid) in 20 ml. 0.1540 molar NaH-d,l-glutamate with 2 % gelatine. Group III did not get any treatment of the transplanted tumors.

In the course of the experiment C a suspicion however arose that by mistake an interchange of the flasks containing the suspensions might have occurred whereby an incontrollable irregularity in the treatment would result. The experiment C consequently was discarded and a new experiment quite similar to experiment C started.

Experiment D. »Crocker 180« was transplanted to 60 Street-mice divided in 3 groups, each of 20. Four days later the treatment began. It was observed that the tumors appeared greater than used to be the case at the start of the treatment. The first injection was exactly as in experiment C but from the second injection the injected volume was increased from 0.2 to 0.35 ml. on account of the exceptional size of the tumors. The animals were given in total 4 injections each with intervals of 2 days. Two days after the last injection the mice were killed and treated as usually. No one had died spontaneously. The results were:

In group I (the l(+)-series)	the average tumor weight was 0.92 g.
» » II (the d, l-series)	» » » » 0.91 g.
» » III (untreated)	» » » » 1.26 g.

In the same manner as in the experiments A and B the tumors which have been treated show a reduction in weight compared with the untreated ones. The reduction was here 27 % and was identical in the series treated with l(+)glutamic and the series treated with d, l-glutamic acid.

This might appear directly contradictory to the hypothesis of Kögl if it is considered a necessary consequence of that hypothesis that a supply of d, l-glutamic acid to the tumor would stimulate its growth. This is, however, not the case.

On account of the very ready racemisation, even in acid solution, of certain others of the amino acids of the proteins (serine, cystine, proline and others) it has not been possible up to now to decide with certainty whether or not the racemic form of these acids are present in larger quantities in hydrolysates from tumor tissues than in hydrolysates from normal tissues. Kögl, therefore, did not make definite conclusions with respect to the eventual importance of these for the structure of tumors but only pointed out the strongly predominating occurrence of d, l-glutamic acid as a main condition for malignant growth. It is still unknown if perhaps simultaneous action of one or more other d-forms is a necessary supplement for the formation of tumor tissue. From a general biological point of view such conjoint action might be the more probable. The result of experiment D seems at any rate to indicate that a supply of d, l-glutamic acid alone is not sufficient to increase the tumor growth, while on the other hand the abundant supply of l(+)glutamic acid in all experiments in this paper seems to have produced the desired alteration in the pathological

protein synthesis. It is also of interest to notice that $l(+)$ glutamic acid has had no toxic effect in any of the experiments.

To get a chance of deciding by statistical treatment the trustworthiness or the more or less occasional character of the results won in the present investigation the previously mentioned measurements of the size of the tumors were made. For the experiments A, B and D they are given in the tables III—IX. The necessary condition for getting trustworthy results from a statistical treatment of the observed values of the tumor size is, as it is well known, that the variations are in agreement with Gauss' distribution law (normal distribution). To be able to judge of whether this is the case or not the so called Probit-method was applied. The essence of this method is that a straight line will result in case of normal distribution when the varying values of the «random-variable», here the volumes of the tumors, are plotted against the so called Probit cipher found in a special Probit-table¹¹⁾ by means of the percentage occurrence of the »random variable«. The volume of a tumor was taken as equal to an ellipsoid with axes equal to the three dimensions measured. For all 7 series (2 in experiment A, 2 in B and 3 in D) the Probit method however gave curved lines showing thereby that the tumor volumes themselves are not normally distributed. In biological investigations however it often happens that it is not the directly measured variable which is normally distributed but a certain function of this variable, not seldom the logarithm; therefore this transformation was tried. The application of the Probit method to the logarithm of the tumor volumes however gave curves which curved in the opposite direction of the above mentioned curves. As a consequence a transformation had to be sought which would act in the same direction as the logarithm but less strongly. Dr. G. Rasch of the Royal Serum Institute, Copenhagen, with whom I discussed the case kindly agreed to undertake the mathematical-statistical analysis of the results and I am indebted to Dr. Rasch for the solution of the special difficulties which this case exhibited. As a transformation, which might perhaps have a biological interpretation, the distribution of the cubic root of the tumor volume was investigated and showed to give satisfactory results i. e. probit curves which were straight lines in six of the experiments, namely the two experiments A, the control experiment B and the three experiments D (Tables X—XVI) respectively. The experiment B, $l(+)$ which to a certain degree forms an exception will be discussed in the following. In the above mentioned six experiments the distribution of the cubic root of the tumor volumes consequently may be regarded as normal and the mean value and standard deviation therefore were calculated in the ordinary way giving the results of table I.

In an investigation of mainly orientating character, as the present, it may be considered a sufficient argument for the continuation and

Table I.

Experiment No.	Number of mice	Mean value of $\sqrt[3]{\bar{v}}$	Degrees of freedom	Variance s^2
A. l(+)	21	1.034	20	0.0921
A. control.	17	1.048	16	0.0958
B. control.	20	0.687	19	0.0715
D. l(+)	20	0.966	19	0.0359
D. d,l	20	0.866	19	0.0411
D. 0	19	0.980	18	0.0291

enlargement to a greater scale of the here made experiments, if the difference $(\bar{x}_2 - \bar{x}_1)$ between the mean value \bar{x}_1 of the tumor weights in a series where the tumors were treated with l(+)glutamic acid and the mean value \bar{x}_2 of the tumors in the corresponding untreated control series is twice or more than twice the standard error e_D of the mean values i. e. if

$$t = \frac{\bar{x}_2 - \bar{x}_1}{e_D} \geq 2$$

this value of t meaning, as is well known, that there is only a risk of 5 per cent. that the difference should be due to random variation only.

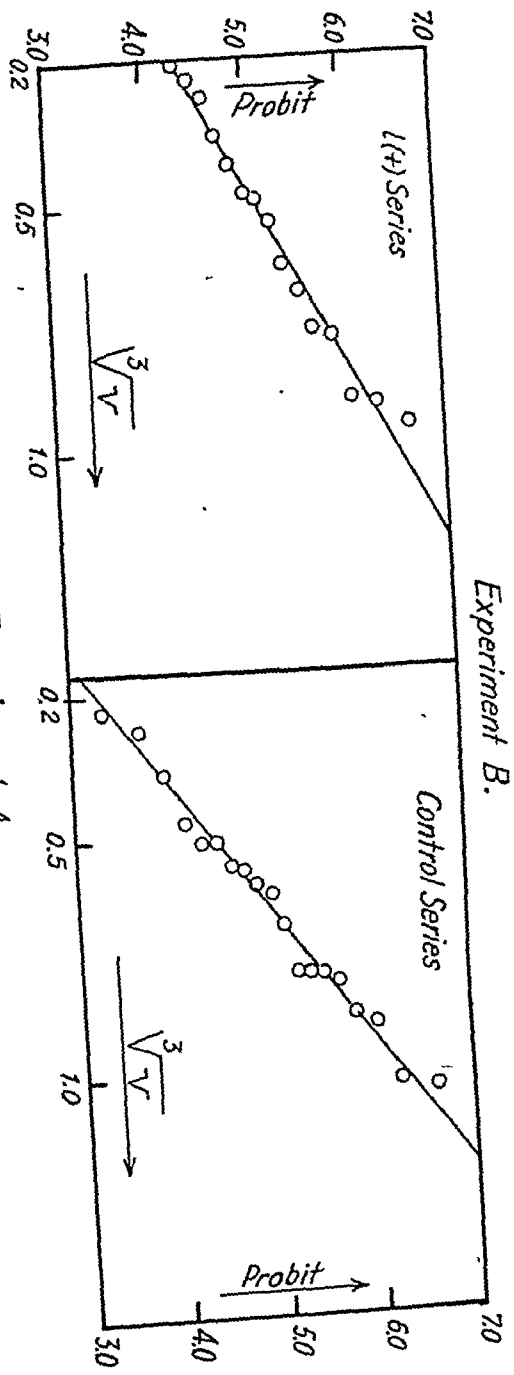
The calculation of t in experiment A and D gives the following values:

Table II.

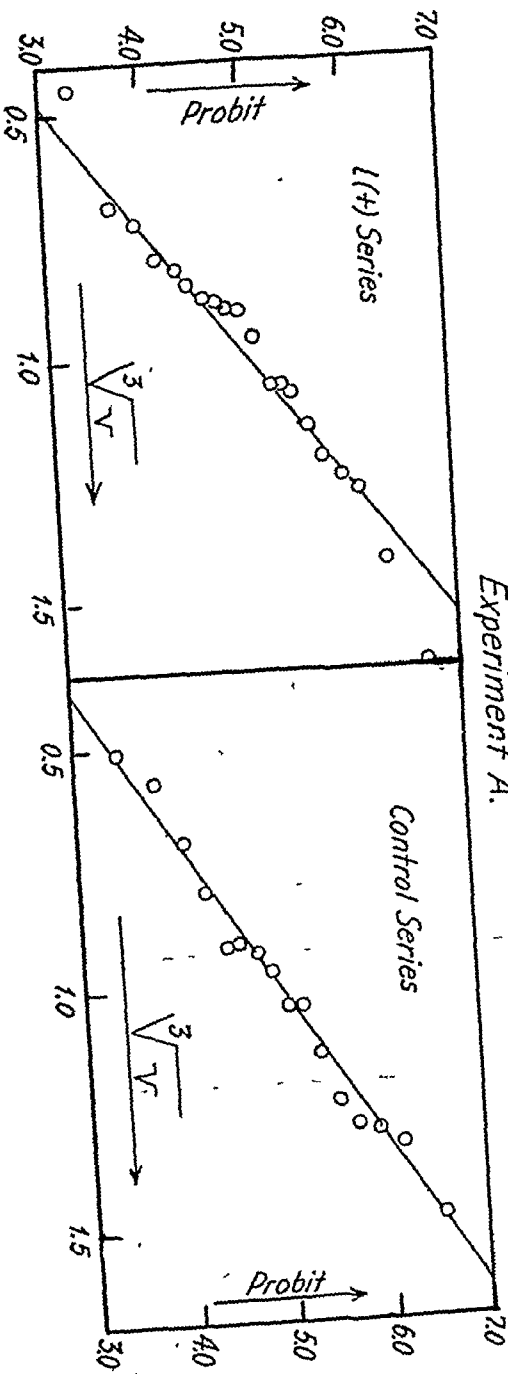
Experiment	$\bar{x}_2 - \bar{x}_1$	e_D	t
A. l(+) — A. control	0.014	0.0999	0.14
D. l(+) — D. d,l	0.100	0.0594	1.68
D. l(+) — D. 0	0.014	0.0603	0.23
D. d,l — D. 0	0.114	0.0603	1.89

It is seen from the values of t that none of the differences are significant. The only possibility of pointing out that there may be a reality underlying the reductions of the tumors treated with l(+)glutamic acid in this investigation therefore must be sought in experiment B. Here 4 out of 20 tumors in the l(+) series have completely disappeared. But this fact produces a special difficulty, since the distribution in this series can not be considered as normal. The probit curve for B.l(+), although being a straight line for so long it goes, is only what may be called statistically a half-line which radiates from the probit value corresponding to the 20 per cent. of tumors. If we want to do so, we may imagine the curve completed

Experiment B.



Experiment A.



with the ordinate axis from the said Probit value to $\div \infty$. This type of curve is characteristic for the so-called »truncated normal distribution« (described by *Hald, Jersild and Rasch*¹²) i. e. a normal distribution cut short at one end and where the probability corresponding to the area which has been cut away is placed in the point of interception.

The truncation corresponding to the complete disappearance of the tumors may be explained as follows. It may be assumed that the tumors possess a certain resistance against the treatment and that their distribution according to resistance is normal. The tumors whose resistance lies below a certain value will disappear completely by the treatment while the others will undergo more or less reduction. The distribution of the tumors according to resistance therefore will interfere with the distribution according to their size at the beginning of the treatment and thereby that type of distribution is created of which we have seen a sample in experiment B,1(+) and which can be described as a normal distribution of $\sqrt[3]{V}$, cut short at the zero point.

The ordinary way of estimating the statistical parameters being inadequate in this experiment, the statistical analysis must aim at finding a legitimate estimation of the mean value \bar{x}_1 and the standard deviation s_1 in the truncated normal distribution and an adequate method for the comparison with the mean value \bar{x}_2 and the standard deviation s_2 in the control experiment B. This can be done by means of special mathematical-statistical methods which will be given in a coming paper: *A. Hald*¹³): »The truncated normal distribution«. Thereby, for the experiment B,1(+), the following values are found:

$$\bar{x}_1 = 0.452 \text{ and } s_1 = 0.442$$

which, combined with the values given in table I for experiment B, control:

$$\bar{x}_2 = 0.687 \text{ and } s_2 = 0.267 (= \sqrt{0.0715}),$$

by a modification of the ordinary t-test, give the value:

$$t = 2.10$$

a value which lies a little above the 5 per cent. limitation.

Experiment B,1(+) consequently strongly advocates a continuation and extension of the present investigation.

The better results of the tumor treatment in B,1(+), than in the other experiments of this paper, are in harmony with the experimental conditions, since it may be assumed that the purpose of the tumor treatment i. e. the insulation of the cancer tissue from an eventual supply of tumorfavouring substances and at the same time the production of a large excess of l(+)glutamic acid will have the better

chances of becoming realised the larger the injected dose and the smaller the tumor i. e. the shorter the time which elapses between the transplantation of the tumor and the beginning of the treatment.

By the influence of substances foreign to the organism (as distinct from hormones, enzymes and other substances extracted from organisms, the effect of which upon cancer has often been investigated but shall not be mentioned here) several scientists¹⁴⁾ have succeeded in producing reduction or even disappearance of tumors, mainly experimental animal tumors. Among the many different and differently applied chemicals may be mentioned aldehydes, ketones, diamines, sulfamides and, what would seem to be of special interest, carcinogenic substances. Among the several cases the treatment of 22 human patients by *K. H. Bauer*¹⁵⁾ with the strongly carcinogenic benzpyrene is worthy of notice. Seven of the twentytwo cancer patients were cured and had, when the result was published, been well for two years.

As is well known tumor cells in comparison to normal cells have only a short life-time and show slight resistance against restraining influences of different kind. Of this the Roentgen-therapie is the commonly known example. The tumor decreasing action of the applied chemicals named above hardly can be considered as having been of specific nature but probably only has been due to the general growth-disturbing action of these more or less injuring or noxious substances.

The present investigation has not solved the question concerning the correctness of Kögl's hypothesis, in spite of the unmistakable connection between the tumor-growth and the presence of an excess of l(+)glutamic acid seen in the here made experiments. To decide whether or not this result means a support for Kögl's views it will probably be necessary to give that hypothesis in a more elaborate form, taking account of more factors than the presence of d(÷)-glutamic acid only as a condition for malign cell-growth.

Apart from the relation to Kögl's hypothesis the experimental results of the present paper have shown that, as distinct from the above cited results won by treatment with more or less noxious substances, reductions of tumors can be caused by l(+)glutamic acid which, according to the observations made here, has acted upon the tumor only and thus, if these results are confirmed by repetition on large scale, has shown to be a substance of specific tumor-reducing effect. Being at the same time a substance occurring in the sound organism as a constituent part of normal proteins and present in more or less free state in the circulation, l(+)glutamic acid may be expected to show noxious effects in exceptional cases only. That transplanted tumors, however, often are reacting in another way than spontane tumors will do, scarcely needs to be mentioned here.

The present paper, however must be considered only as a basis for further investigations on a larger scale, taking in consideration

the experiences won and the reflexions caused by the present research. The technique used here certainly has obvious drawbacks which, however, it may be possible to remove to some degree. As for the cultivation of cell-tissues in vitro which, for obvious reasons, immediately presents itself as an alternative for the investigation of the questions treated here, it might be said that experiments made with the whole living organism will offer the advantage that the cancer-tissue in question will remain, by this method, under the general regulative influence of the entire organism during the whole treatment. That the reactions of the living cells in the intact organism may be qualitatively different from the reactions in vitro has been repeatedly pointed out and, lately, by A. Fischer¹⁶⁾ in an interesting review of some of his own results. From that paper the following is quoted: »Nach Rose gehört also Cystin zu den entbehrlichen Säuren. Dies gilt aber nur für den intakten Organismus, nicht für eine Reihe isolierter Gewebezellen, die ihrerseits das Methionin nicht ausnutzen können. — — — Es ist bekannt, dass isolierte Gewebe anders arbeiten als der Gesamtorganismus.«

Among the factors which according to the introductory character of the present investigation have been put off for the present may be mentioned, on the physiological side, especially the investigation of into what degree a tumor reduced by the treatment with l(+)-glutamic acid has kept its malignant character, its ability to take and to grow malignantly by renewed transplantation to sound animals and to metastasize, as well as the treatment of other tumors, especially, if obtainable, spontaneous tumors. On the technical side: systematic alterations in the medium of suspension and in the magnitude of the dosis; estimation of the most favorable length of the treatment and, especially, changes in the application method e. g. to the use of intratumoral injections together with or without simultaneous subcutane injections.

Finally I wish to express my sincere thanks to Prof. Dr. med. Jul. Engelbreth-Holm for the interest and kindness with which he accepted my idea and gave his permission to have it carried out in the laboratory of the Institute of pathological anatomy, where he placed both material and assistance to my disposal and for the great help and encouragement which our conversations on the subject have been to me.

I also owe sincere thanks to Prof. Anker Engelund, Director of the Royal Polytechnic Institute, for the obligingness with which dispensation for certain instructional duties were granted to me, to the Laurits Andersen Foundation for economic support and to Dr. techn. J. Møller for the preparation of a part of the d,l-glutamic acid employed in the experiments.

Table III.
Experiment A. 1(+).

Mouse No.	Weight	Sex	Tumor	$V = \frac{\pi}{6} \cdot d_1 \cdot d_2 \cdot d_3$
2808	21	F	26×15×15	3.067
—13	26	-	19×15.5×13.5	2.082
—17	27	-	11×13.5×8	0.622
—24	20	-	14×7×10	0.513
—25	20	-	16×9×45	0.339
—39	25	-	23×15×5	0.903
—47	25	-	34×18×14	4.486
—59	28	-	65×6×45	0.092
—60	25	-	15×18×9	1.273
—64	20	M	12×14×8	0.704
—65	24	F	13×10×8	0.545
—70	24	-	23×8×7	0.674
—74	24	M	18.5×11.5×13.5	1.504
—75	24	F	21×15.5×10.5	1.790
—79	27	M	13×12×9	0.735
—84	27	F	13×16×18	1.961
—89	17	M	15×15×10	1.179
—90	27	-	15×12×8	0.754
—91	25	F	26×19×17	4.396
—98	27	-	11×11×6	0.380
2899	23	-	23×10.5×95	1.203

Table IV.
Experiment A. control.

Mouse no	Weight	Sex	Tumor	$V = \frac{\pi}{6} \cdot d_1 \cdot d_2 \cdot d_3$
2811	27	F	10×5×5	0.131
—21	21	-	10.5×7×5	0.192
—26	30	M	23×14×14	2.360
—27	24	-	11.5×15×9	0.813
—33	28	F	27×17.5×18	4.452
—41	27	M	20.5×13×10.5	1.465
—42	23	F	20×11×8	0.922
—43	23	-	15×13×11	1.123
—48	24	M	26×15×16	3.268
—49	25	-	27×14×11	2.177
—50	23	F	17.5×11×11	1.109
—56	18	-	29×14×10	2.126
—77	25	-	14×95×5	0.348
—78	20	-	16.5×11×8	0.760
—88	26	-	15×11×6	0.518
2900	24	-	20×15×12	1.885
—01	21	-	16.5×11×8	0.760

Table V.
Experiment B. 1(+).

Mouse No.	Weight g	Sex	Tumor mm.	$V = \frac{\pi}{6} \cdot d_1 \cdot d_2 \cdot d_3$ cc.
3445	19	M	20×9×8	0.754
—46	19	-	6×5×3	0.047
—48	17	-	18×8×6	0.452
—50	18	-	0	0
—53	18	F	0	0
—55	19	M	22×15×13	2.401
—57	21	F	14×12×10	0.880
—58	19	M	14×4×4	0.117
—59	18	-	3×3×3	0.014
—60	15	-	13×5×7	0.238
—65	17	-	7×7×6	0.154
—67	19	F	12×19×2	0.113
—68	17	-	7×6×1	0.022
—70	19	M	16×11×8	0.737
—71	17	-	0	0
—75	19	-	10×10×6	0.314
—77	17	F	11×15×5	0.432
—79	17	M	0	0
—80	15	-	3×3×2	0.009
3482	14	F	10×5×3	0.078

Table VI.
Experiment B. control.

Mouse No.	Weight g	Sex	Tumor mm.	$V = \frac{\pi}{6} \cdot d_1 \cdot d_2 \cdot d_3$ cc.
3447	18	M	18×9×6	0.535
—49	15	F	7×12×4	0.176
—51	19	M	14×18×8	1.051
—52	17	-	8×6×2	0.050
—54	17	F	11×7×8	0.323
—56	24	M	14×10×10	0.733
—61	21	-	12×10×8	0.503
—62	20	-	18×13×9	1.120
—63	17	-	10×7×6	0.220
—64	15	F	8×7×6	0.134
—66	20	M	10×5×5	0.131
—69	21	F	15×9×7	0.495
—72	20	M	3×3×4	0.013
—73	19	-	12×4×4	0.103
—74	20	F	23×14×13	0.193
—76	17	M	11×12×7	0.484
—78	18	F	12×12×9	0.679
—81	18	M	5×4×2	0.021
—83	19	F	8×9×5	0.189
3484	19	-	9×10×5	0.236

Table VII.
Experiment D.1(+).

Mouse No.	Weight g.	Sex	Tumor mm.	$V = \frac{\pi}{6} \cdot d_1 \cdot d_2 \cdot d_3$ cc.
40795	20	M	20×17×16	1.780
—96	18	-	11×8×10	0.461
—97	18	-	9×8×6	0.226
—98	19	-	15×12×10	0.943
—99	19	-	22×12×12	1.569
40800	20	-	16×18×11	1.693
—01	10	-	8×12×12	1.357
—02	16	-	22×13×13	1.946
—03	20	-	14×9×8	0.528
—04	18	-	15×14×10	1.100
—05	19	-	17×10×10	0.891
—06	24	-	20×11×15	1.748
—07	21	-	18×11×12	1.244
—08	21	-	7×10×8	0.721
—09	23	-	18×12×6	0.679
—10	20	-	15×14×9	0.996
—11	23	-	16×14×6	0.704
—12	17	-	15×10×8	0.628
—13	20	-	15×9×7	0.495
40814	15	-	10×10×5	0.262

Table VIII.
Experiment D. d,1.

Mouse No.	Weight g.	Sex	Tumor mm.	$V = \frac{\pi}{5} \cdot d_1 \cdot d_2 \cdot d_3$ cc.
40815	20	M	10×8×5	0.210
—16	23	-	16×11×6	0.553
—17	17	-	15×13×8	0.817
—18	18	-	21×12×11	1.452
—19	10	-	13×12×9	0.735
—20	22	-	14×9×10	0.660
—21	24	-	8×7×5	0.147
—22	22	-	12×9×8	0.452
—23	18	-	14×12×9	0.792
—24	14	-	13×15×8	0.817
—25	15	-	19×12×9	1.074
—26	17	-	16×8×6	0.402
—27	17	-	17×14×7	0.872
—28	17	-	11×9×7	0.363
—29	15	-	20×15×12	1.885
—30	24	-	17×13×12	1.389
—31	18	-	10×6×5	0.157
—32	18	-	20×12×10	1.257
—33	19	-	12×6×6	0.226
40834	20	-	13×11×9	0.674

Table IX.
Experiment D. 0.

Mouse No.	Weight g.	Sex	Tumor mm.	$V = \frac{\pi}{6} \cdot d_1 \cdot d_2 \cdot d_3$ cc.
40835	19	M	16×12×7	0.704
—36	18	-	10×8×7	0.586
—37	17	-	21×12×9	1.188
—38	25	-	17×13×10	0.356
—39	20	-	17×11×8	0.703
—40	17	F	6×6×5	— *)
—41	16	-	13×9×8	0.490
—42	20	-	12×14×14	1.231
—43	14	-	16×13×10	1.089
—44	17	-	19×16×14	2.228
—45	17	-	17×12×8	0.807
—46	17	-	15×10×10	0.785
—47	20	-	15×8×9	0.649
—48	20	-	15×10×10	0.785
—49	19	-	15×15×9	1.060
—50	16	-	15×17×13	1.736
—51	19	-	14×10×10	0.733
—52	13	-	20×11×12	1.382
—53	15	-	14×10×8	0.586
40854	18	-	25×11×8	1.152

*) Tumor infected.

Table X.
Experiment A. 1(+).

Mouse No.	%	Probit	$\sqrt[3]{V}$
1 2859	4.76	3.33	0.452
2 —25	9.52	3.69	0.697
3 —98	14.29	3.93	0.724
4 —24	19.05	4.12	0.800
5 —65	23.81	4.29	0.817
6 —17	28.57	4.43	0.853
7 —70	33.33	4.57	0.877
8 —64	38.10	4.70	0.889
9 —79	42.86	4.82	0.902
10 —90	47.62	4.94	0.910
11 —39	52.38	5.06	0.966
12 —89	57.14	5.18	1.06
13 —99	61.91	5.30	1.06
14 —60	66.67	5.43	1.08
15 —74	71.43	5.57	1.15
16 —75	76.19	5.71	1.21
17 —84	80.95	5.88	1.25
18 —13	85.72	6.07	1.28
19 —08	90.48	6.31	1.45
20 —91	95.24	6.67	1.64
21 —47	100.00	—	1.65

Table XI.
Experiment A. control.

Mouse No.	%	Probit	$\sqrt[3]{V}$
1 2811	5.88	3.33	0.508
2 —21	11.76	3.81	0.577
3 —77	17.65	4.07	0.703
4 —88	23.53	4.28	0.804
5 —78	29.41	4.46	0.912
6 2901	35.29	4.62	0.912
7 2827	41.18	4.78	0.933
8 —42	47.06	4.93	0.973
9 —50	52.94	5.07	1.04
10 —43	58.82	5.22	1.04
11 —41	64.71	5.38	1.14
12 2900	70.59	5.54	1.24
13 2856	76.47	5.72	1.29
14 —49	82.35	5.93	1.30
15 —26	88.24	6.19	1.33
16 —24	94.12	6.57	1.48
17 —33	100.00	—	1.64

Table XII.
Experiment B. 1(+).

Mouse No.	%	Probit	$\sqrt[3]{v}$
1 3450	5.00	3.36	0.000
2 —53	10.00	3.72	0.000
3 —71	15.00	3.96	0.000
4 —79	20.00	4.16	0.000
5 —80	25.00	4.33	0.208
6 —59	30.00	4.48	0.241
7 —68	35.00	4.61	0.280
8 —46	40.00	4.75	0.361
9 —82	45.00	4.87	0.428
10 —67	50.00	5.00	0.483
11 —58	55.00	5.13	0.489
12 —65	60.00	5.25	0.536
13 —60	65.00	5.39	0.619
14 —75	70.00	5.52	0.679
15 —77	75.00	5.67	0.757
16 —48	80.00	5.84	0.767
17 —70	85.00	6.04	0.904
18 —45	90.00	6.28	0.910
19 —57	95.00	6.64	0.957
20 3455	100.00	—	1.34

Table XIII.
Experiment B. control.

Mouse No.	%	Probit	$\sqrt[3]{v}$
1 3472	5.00	3.36	0.235
2 —81	10.00	3.72	0.276
3 —52	15.00	3.96	0.368
4 —73	20.00	4.16	0.469
5 —66	25.00	4.33	0.508
6 —64	30.00	4.48	0.512
7 —49	35.00	4.61	0.561
8 —83	40.00	4.75	0.574
9 —63	45.00	4.87	0.604
10 —84	50.00	5.00	0.618
11 —54	55.00	5.13	0.686
12 —76	60.00	5.25	0.785
13 —69	65.00	5.39	0.791
14 —61	70.00	5.52	0.796
15 —47	75.00	5.67	0.811
16 —78	80.00	5.84	0.879
17 —56	85.00	6.04	0.902
18 —51	90.00	6.28	1.02
19 —62	95.00	6.64	1.04
20 —74	100.00	—	1.30

Table XIV.
Experiment D. 1(+).

Mouse No.	%	Probit	$\sqrt[3]{v}$
1 40797	5.00	3.36	0.610
2 —814	10.00	3.72	0.640
3 —796	15.00	3.96	0.773
4 —813	20.00	4.16	0.791
5 —03	25.00	4.33	0.809
6 —12	30.00	4.48	0.857
7 —09	35.00	4.61	0.879
8 —11	40.00	4.75	0.889
9 —08	45.00	4.87	0.898
10 —05	50.00	5.00	0.962
11 40798	55.00	5.13	0.980
12 —810	60.00	5.25	0.998
13 —04	65.00	5.39	1.03
14 —07	70.00	5.52	1.08
15 —01	75.00	5.67	1.11
16 40799	80.00	5.84	1.18
17 —800	85.00	6.04	1.18
18 —806	90.00	6.28	1.20
19 —795	95.00	6.64	1.21
20 —802	100.00	—	1.25

Table XV.
Experiment D. d,1.

Mouse No.	%	Probit	$\sqrt[3]{v}$
1 40821	5.00	3.36	0.527
2 —31	10.00	3.72	0.540
3 —15	15.00	3.96	0.594
4 —33	20.00	4.16	0.610
5 —28	25.00	4.33	0.713
6 —26	30.00	4.48	0.738
7 —22	35.00	4.61	0.767
8 —16	40.00	4.75	0.820
9 —20	45.00	4.87	0.871
10 —34	50.00	5.00	0.877
11 —19	55.00	5.13	0.902
12 —23	60.00	5.25	0.925
13 —17	65.00	5.39	0.935
14 —24	70.00	5.52	0.935
15 —27	75.00	5.67	0.955
16 —25	80.00	5.84	1.02
17 —32	85.00	6.04	1.08
18 —30	90.00	6.28	1.12
19 —18	95.00	6.64	1.13
20 —29	100.00	—	1.24

Table XVI.
Experiment D. 0.

Mouse No.	%	»Probit«	$\sqrt[3]{v}$
1 40838	5.26	3.38	0.710
2 —41	10.53	3.75	0.789
3 —36	15.79	4.00	0.838
4 —53	21.05	4.19	0.838
5 —47	26.32	4.37	0.865
6 —39	31.58	4.52	0.889
7 —35	36.84	4.66	0.889
8 —51	42.10	4.80	0.902
9 —46	47.37	4.93	0.923
10 —48	52.63	5.07	0.923
11 —45	57.89	5.20	0.931
12 —49	63.16	5.34	1.02
13 —43	68.42	5.48	1.03
14 —54	73.68	5.63	1.05
15 —37	78.95	5.80	1.06
16 —52	84.21	6.00	1.11
17 —50	89.47	6.25	1.20
18 —44	94.74	6.62	1.31
19 —42	100.00	—	1.35

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ON THE GENESIS OF DIVERTICULUM DUODENI*)

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(Received for publication January 28th, 1944).

The etiology and genesis of the duodenal diverticula must be said still to be rather obscure. This is evident, for one thing, from the great number of possible causes of this phenomenon given in the literature.

Boyd (1938) distinguishes between primary and secondary diverticula. A *primary diverticulum* usually arises in the second part of the duodenum, sometimes in the first, rarely in the third. It issues from the inner and posterior aspects of the duodenum along the line where the blood vessels enter and weaken the wall. It consists in a hernia-like pouching of the mucosa through the muscularis, varying in size from that of a pea to a plum. Diverticula may be single or multiple and are found in middle-aged and elderly persons.

Secondary diverticula are secondary to duodenal ulcers and are found in the first part of the duodenum.

Cole & Roberts (1920) have reviewed the previous literature on the subject and reported 30 cases of duodenal diverticulum diagnosed by roentgenography, giving as etiological factors, besides ulcer: traction from the outside, resulting from atrophy of the pancreas (Roth, 1872) shrinkage after cholecystitis, and pressure from partial obstruction of the duodenum below the diverticula. Further, the diverticula are said sometimes to be congenital, representing »abortive anlage of a supernumerary pancreas« in the sense that this pancreatic anlage causes local defects in the musculature, whereafter factors as advancing age, lack of tonus or increase in the intraduodenal pressure induce the production of the diverticulum. According to Cole & Roberts a little pancreatic tissue is often present in the duodenal mucosa, but Brunner glands are absent. Of other factors which may promote a gradual protrusion of the mucosa, Roth mentions abnormal laxity of the duodenum and fatty degeneration of the tunica muscularis.

Testut (1931) states that the diverticula in the second part of the duodenum most often are located in the region of the ampulla of Vater. They extend towards the pancreas anterior and posterior to the gland, and they may make their way into the pancreatic parenchyma. The pathogenesis is still

*) Translated from Danish by Hans Andersen M.D.

rather obscure. In some cases the diverticulum unquestionably represents a pathological condition (a result of tuberculosis or of peptic ulcer), but such cases are exceptions. The majority result from a congenital malformation or sometimes from an acquired weakness. The penetration of the muscularis by the veins gives rise to zones of lower resistance, which may be the origin of »hernies des tuniques« if these are altered, if the intestine is distended, or if the veins are dilated.

Embryology explains in part the etiology of certain diverticula. At a given point of time in intrauterine life (length of fetus 10–20 mm.) the originally empty duodenal tube undergoes an epithelial occlusion resulting from proliferation of the epithelium. After this, the mesenchyma round the duodenum is the site of intensive growth. The epithelial wall is hollowed by lacunae, and the mesenchyma makes its way into the intervals between these lacunae. The lacunae unite, and the central lumen makes its appearance (30 mm. embryo). At this developmental stage diverticular formations have been observed in the epithelial wall of the duodenum, and they are said to be constant phenomena in mammalian embryos. Consequently, some of the diverticula observed in adults have been assumed to represent the persistence and accentuation of such a normal embryonal stage. Thus a developmental disturbance must have been present, a disharmony between the development of epithelial tissue and mesenchymal, a delay in the appearance of the latter, or an accentuated development of diverticular epithelial proliferation. Testut does not acknowledge the division of the diverticula which considers a diverticulum congenital when its wall includes all layers of the duodenal wall, and as acquired when its wall is incomplete. The diverticula represent a »prenatal« affection, not a »post-natal«.

Since the introduction of X-ray examination of the gastro-intestinal tract the interest in duodenal diverticula has been increasing. Among recent publications in this country mention may be made of the studies reported by Jens Juul (1929), H. Brammer (1943), Hjordis Jørgensen (1943) and T. Thune Andersen & Sv. Erik Nielsen (1943), dealing chiefly with the roentgenological and clinical aspects of the duodenal diverticula, whereas no independent investigation into the genesis of the phenomenon has been published.

Writer's Studies.

In a material comprising 53 duodena employed for studies on the distribution and quantity of the Brunner glands in man (Landboe-Christensen, 1944) duodenal diverticula were found in 4 cases. This material represented a »serial material« in so far as no other selection took place than omission of specimens with coarse pathological changes that could be diagnosed on the macroscopic routine examination on the autopsy table. In all 4 cases therefore the diverticula were so small that they were diagnosed only at the subsequent, more thorough, anatomical examination of each specimen. The specimens were unfixed total preparations stained after the method given by Landboe-Christensen (1944) for staining of the duodenal glands in preparation of the entire duodenum.

Examination of these diverticula showed certain interesting features, and as these appear to be able to contribute to the understanding of

the genesis of at least certain diverticula, it will be appropriate here to mention them somewhat in detail.

Duodenum No. 1 (Autopsy No. 184/41).

Woman, aged 49. Cause of death: Softening of the brain.

The posterior wall of the duodenum is the site of 3 small diverticula — 2 of about nut kernel size, 1 almost of pea size — located respectively in the interpapillary section of the duodenum (2 cm. proximally to the inferior papilla) and in the infrapapillary section (3 and 13 cm. below the inferior papilla). All the diverticula lie on the same line, exactly at the entrance of the dorsal duodenal vessels into the submucosa.

Duodenum No. 2 (Autopsy No. 235/41).

Woman, aged 65. Cause of death: Pulmonary embolism.

In the infrapapillary section of the duodenum (1½ cm. proximally to the commencement of the mesentery) a diverticulum, size of nut kernel is located at the entrance of the dorsal vessels into the submucosa.

Duodenum No. 3 (Autopsy No. 177/41).

Man, aged 75. Cause of death: Oedema of the meninges; arterial hypertension.

About 1 cm. proximally to the commencement of the mesentery, the posterior wall of the duodenum presents a nearly pea-sized diverticulum at the entrance of the dorsal vessels into the submucosa. At the »bottom« of the diverticulum, the submucosal connective tissue extends along the vessels as a solid »tuft«, the presence of which presumably can be due only to traction exerted on the submucosal tissue.

Duodenum No. 4 (Autopsy No. 194/41).

Man, aged 83. Cause of death: Softening of the brain.

In the suprapapillary section of the duodenum (2 cm. proximally and ¾ cm. dorsally to the superior papilla) a diverticulum, a little larger than a nut kernel is seen at the entrance of the dorsal vessels into the submucosa. The same specimen shows 1 cm. distally to the commencement of the mesentery — *i. e.*, in the jejunum — an elongated polyp, 0.5 × 1 cm., with a smooth surface covered by mucosa. The polyp is situated at the entrance of the dorsal vessels into the submucosa.

Here then we have 4 cases of small duodenal diverticula, 3 with a solitary diverticulum, 1 with multiple. The diverticula are demonstrated in various parts of the duodenum — supra-, inter- and

infrapapillary, and at the commencement of the mesentery — but in every instance at the entrance of the dorsal vessels into the submucosa and in parts of the duodenum which are in close relation to the pancreas.

On correlation of the particular location of the diverticula and the presence of the above-mentioned phenomenon of traction (duodenum No. 3) with certain particular aspects of the normal anatomy of the duodenum and with the shifts taking place in the duodenal wall under peristalsis, it seems practicable to reach an understanding of the genesis of the diverticula here concerned.

The fixation of the pancreas to the duodenum on the whole is tight, especially at, above and below the interpapillary section (which is readily ascertained by separation of the pancreatic tissue from the duodenum in an unfixed specimen). The arterial branches to the duodenum (*rami duodenales*) arise at the level of »the pancreatic part« from the »arcades« situated ventrally and dorsally between the head of the pancreas and the duodenum, passing then with their main trunks accompanied by large veins, through the tunica muscularis and entering quickly the submucosa. So the origins of the submucosal vessels lie fixed anteriorly and posteriorly between the pancreas and the tightly bound duodenum. On movements of the gut (contraction, dilatation) the individual layers of the intestinal wall are shifting in their mutual relation. The shift of the mucosa in relation to the muscularis is particularly pronounced — *i. e.*, the shift in the loose-woven *tela submucosa*.

Through this shift, now, a traction is exerted on the submucous vessels, and naturally this traction is greatest at the transition between the fixed part of the vessels and the unfixed, movable, part. At the sites of perforation the submucosal connective tissue is relatively fixed, (through the vessels), whereas in other places the submucosal tissue will be pulled this way and that way under the movements of the gut. So it appears as if the intestinal movements through traction and counter traction imply the possibility of the formation of a submucosal »tuft« at the entrance of the vessels, leading gradually to mucosal pouching and formation of the diverticulum. Probably the diverticulum formation is also promoted by the fact that the sites of perforation naturally have to be looked upon as the weak spots in the intestinal wall which gradually may yield to the internal pressure.

So the genesis of the duodenal diverticula mentioned here appears to be attributable to traction in connection with the particular anatomic-topographical peculiarities of the duodenum. For the sake of comparison it may be mentioned that diverticulum of the colon, which likewise is a phenomenon of advancing age, is interpreted as brought about by the naturally occurring »hernial canals« — the vascular perforations through the muscularis of the gut — in connection with

internal pressure (*i. e.*, pulsion). Stasis in the intestinal veins is said to promote the appearance of the diverticula (for details see the monograph by Hans Thomsen (1934) in which a review is given of the previous literature on diverticula of the colon). — Finally it is to be mentioned that the statement made by Cole & Roberts about Brunner glands not being present in the duodenal diverticula, does not agree with observations made by the writer. In so far as the diverticula are located within the area covered by Brunner glands in the specimen concerned, Brunner glands are present in these diverticula too — at any rate in diverticula of the same size as those described in this paper.

Summary.

A survey is given of the possible causes of duodenal diverticulum as mentioned in the literature.

In a »serial material« comprising 53 duodena (autopsy specimens) without gross pathologic-anatomical changes, the writer ascertained in four specimens the presence of duodenal diverticula so small that they had escaped demonstration at the routine examination of the gut on the autopsy table.

From correlation of certain special features in the normal anatomy of the duodenum with the shifts taking place in the duodenal wall under movements of the intestine (traction) it appears practicable to explain the genesis of the diverticula here concerned and their particular localization.

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LEPTOSPIRA BALLUM

A NEW SEROLOGICAL LEPTOSPIRA TYPE?

By C. Borg Petersen.

(Received for publication April 17th, 1944).

In the latter part of October 1943, through the kindness of Dr. E. Ulfbeck-Petersen, Skærbæk, the State Serum Institute received a field-house mouse (*Mus musculus spicilegus*), caught on a farm near Ballum, South Jutland, where a case of leptospirosis grippotyphosa had occurred in September 1943.*) The urine of this mouse was found to contain numerous living leptospirae. The strain was isolated in culture by way of cavia, and a rabbit was immunized with this culture, »Mus 127«. On the subsequent serological examination (agglutination and lysis experiments with living cultures) strain »Mus 127« was found to differ from all the other leptospira type strains in the tribarium of the Institute.

Serological Examination of Strain »Mus 127«.

As will be noticed from *Table 1*, strain Mus 127 gave cross reactions with types icterohaemorrhagia A and AB, canicola, sejroe, pyrogenes and naam, whereas it showed no serological relationship with the other types. The cross reactions with the canicola type were the strongest. Absorption experiments (carried out with a technique previously described in this journal, *Borg Petersen*, 1944) showed plainly, however, that the antigen content of the two types differs — as is evident from *Table 2*.

It has not been practicable to compare our strain with the type poi described by *Mino* (1941, 1942). According to *Mino*, poi is serologically related to *L. icterohaemorrhagiae*, whereas nothing is said about its relation to *L. canicola*.

Nor have we been able to compare our strain with strains McIntyre and L. 5260 described by *Gardner & Vincent* (1943). According to

*) From the same farm, 5 additional mice (*M. spicilegus*) were received. In 4 of them, which were dead on their arrival, no leptospira could be demonstrated; in the fifth, which arrived alive, leptospirae of the saxkoebing type were found.

Table 1.

Strain	Serological type	Monovalent rabbit immune serum produced with		
		»Mus 127« own titer: 10,000	Strain in column (a)	
			Own titer	Titer with »Mus 127«
(a)	(b)	(c)	(d)	(e)
R G A	icterohaemorrhagia A	100	3,000	0
M 20	» AB	300	30,000	300
Moskou V	grippotyphosa	0	10,000	0
H. Utrecht IV	canicola	1000	10,000	1000
M 84	sejroe	100	3,000	0
Mus 24	saxkoebing	0	10,000	0
Van Tienen	batavia	0	10,000	0
Pomona	pomona	0	30,000	0
Ballico	australis a	0	10,000	0
Hebdomadis	hebdomadis	0	3,000	0
HC	hc	0	3,000	0
3705	3705	0	10,000	0
Autumnalis	autumnalis	0	3,000	0
Rachmat	rachmat	0	10,000	0
Sentot	sentot	0	1,000	0
Djasiman	djasiman	0	10,000	0
Salinem	pyrogenes	0	3,000	100
Zanoni	australis b	0	3,000	0
R. Semarang 173	semarang 173	0	10,000	0
Sarmin	sarmin	0	3,000	0
Naam	naam	300	3,000	0
Vleermuis 90 C	90 c	0	3,000	0
Benjamin	benjamin	0	3,000	0
R. Batavia 46	javanica	0	10,000	0
CH 11	andaman a	0	3,000	0

0 = < 30

Table 2.

Culture. Strain and type.	Monovalent rabbit immune serum produced with			
	»H. Utrecht IV«		»Mus 127«	
	Unabsorbed	Absorbed with »Mus 127«	Unabsorbed	Absorbed with »H. Utrecht IV«
»H. Utrecht IV« (canicola)	3000	3000	300	0
»Mus 127« (ballum)	1000	0	3000	3000

0 = < 100

Gardner & Vincent, however, these strains appear not to be serologically related to *L. canicola*.

With reservation as to the outcome of a future comparison with the strains just mentioned, therefore, we think that for the present we have to look upon Mus 127 as a representative of a serological type not described hitherto, for which we suggest the designation *L. ballum* — after the origin of our strain.

Virulence of L. ballum for Guinea-pig.

From the first guinea-pig inoculated with freshly voided urine from the mouse, the strain was carried through additional 13 guinea-pig passages. As a rule, peritoneal exudate was used for the inoculation, exceptionally ear blood or heart's blood. The inoculation was always performed intraperitoneally; the weight of the guinea-pigs varied from 140 to 170 g.

Without exception, in a few days after the inoculation the guinea-pigs showed the presence of leptospirae in the peritoneal fluid, besides a loss of weight (the temperature was not measured). Of the total 16 inoculated guinea-pigs, 2 survived the infection without showing the least sign of jaundice. The remaining 14 guinea-pigs died 7—12 (on an average 9.2) days after the inoculation. Cultures from the heart's blood of these animals gave in every instance growth of leptospirae in Korthof's culture medium, whereas cultures on blood agar from heart's blood and liver in no case gave growth of bacteria (only aerobic cultivation was employed). So there can hardly be any doubt that the guinea-pigs died of the leptospira infection.

Of these 14 guinea-pigs, 7 showed jaundice — moderate in 2 cases, only slight in 5. Hemorrhages in the adipose tissue and in the organs were generally pronounced but little; and in 2 cases the autopsy revealed no hemorrhages at all. In 9 guinea-pigs, macroscopic changes were found in the kidneys, most often in the form of pronounced hyperemia at the border between the medulla and cortex; 6 of these animals showed also numerous small greyish necrotic foci in the liver. The spleen was generally enlarged, most often but slightly, in a few cases considerably. As a rule, the adrenals were swollen, and more or less hyperemic.

Table 3 gives a survey of the outcome of the guinea-pig inoculation. As will be noticed from this tabulation, there was no distinct increase in virulence in the 14 guinea-pig passages. Still, jaundice was not seen before the 6' passage.

The two surviving guinea-pigs were killed respectively 105 and 36 days after the inoculation. The animal that was killed 36 days after the inoculation showed the presence of leptospirae in the kidneys (growth in cultures), while the other showed no evidence of leptospirae being present.

Table 3.

Guinea-pig passage	Fate of the animal	Hemorrhages	Jaundice	Changes in the liver	Changes in the kidneys	Fate of the animal	Hemorrhages	Jaundice	Changes in the liver	Changes in the kidneys
1.	R									
2.	D ⁹			(N)	(H)					
3.	D ⁸	(+)								
4.	D ¹²	(+)		N	H					
5.	D ⁸	(+)			H					
6.	D ⁷	(+)				D ¹⁰	+	(+)		
7.	D ⁹				P	D ⁸	(+)	(+)		
8.	D ⁸	(+)	(+)							
9.	D ¹⁰	(+)	+	N	H					
10.	D ⁹	+	(+)	N	H					
11.	D ¹¹	+		N	P+H					
12.	D ¹⁰	(+)	+		(P)					
13.	D ¹⁰	(+)	(+)	N	H					
14.	R									

R = recovered. D = died. The small figures give the lifetime after the inoculation, in days.

() indicates that the symptom was pronounced but slightly.

N = necrotic foci.

H = hyperemia of the arcuate zone. P = parenchymatous degeneration. An empty space means that no macroscopic changes were observed.

By causing the death of 88 % of the animals inoculated with this strain, *L. ballum* has shown a virulence for guinea-pigs which approaches markedly that of *L. icterohaemorrhagiae*. On the other hand, the icterogenic properties of *L. ballum* appear to be considerably less pronounced than those of *L. icterohaemorrhagiae*, as jaundice was observed only in 44 % of the animals.

Virulence of L. ballum for White Rat and Mouse.

3 albino rats, 3 weeks old (weighing 45—55 g.) were each inoculated intraperitoneally with 0.1 cc. of citrated heart's blood from a guinea-pig with leptospirosis ballum (guinea-pig passage 13). 3—4 days later, the rats showed a few leptospirae in the peritoneal fluid for 1—2 days, but no noticeable signs of illness. 4 weeks later, the presence of leptospirae in the urine could be demonstrated in all 3 rats by direct microscopy of the urine in dark-field preparations.

3 albino mice, about 5—6 weeks old (weight 13—14 g.) that were inoculated in the same manner, showed leptospirae in the peritoneal fluid for 1—4 days reckoned from the 3' day after the inoculation, but no noticeable external signs of illness, apart from a slight transitory loss of weight. On the 7'—10' day the animals commenced to

excrete leptospirae with the urine; and this excretion is persisting yet at this writing — 4 months after the inoculation.

Thus, *L. ballum* is able to infect young white rats and mice and make them leptospira excreters.*)

Human Infection with L. ballum.

So far, infection with *L. ballum* has not been demonstrated in man. Since January 1st, 1944 strain »Mus 127« has been included in the routine examination of blood specimens for leptospirosis carried out in the institute. Thus, up to April 1st altogether 936 blood specimens have been examined, 16 of which from 9 different patients gave positive reactions from 30 to 1000 with *L. ballum*; but all these specimens showed a higher titer with *L. canicola* or, in two cases, *L. sejroe*. So the reactions obtained with *L. ballum* are most likely to be looked upon merely as joint reactions.

Serum from the patient mentioned in the introduction who had been suffering from leptospirosis grippotyphosa and was living on the farm in Ballum from which the mouse originated, showed no reaction whatever with *L. ballum*.

Summary.

From the urine of a mouse (*Mus musculus spicilegus*) a leptospira strain, »Mus 127« is isolated and found to differ serologically from all the serological leptospira types established so far, perhaps with exception of *Mino's* type poi and the two English strains described by *Gardner & Vincent*, with which it could not be compared under the present conditions. As the preliminary name for the type represented by »Mus 127«, *L. ballum* is suggested — after the place where this strain originated from.

The virulence of *L. ballum* for young guinea-pigs is of the same magnitude as that of *L. icterohaemorrhagiae*, but jaundice occurs considerably less frequently in leptospirosis ballum than in leptospirosis icterohaemorrhagica.

The virulence of *L. ballum* for young white rats and mice appears to be only slight, but so far only a few experiments of this kind have been made. The infected animals became leptospira carriers.

Infection of man with *L. ballum* has not been ascertained so far.

*) The field-house mouse from which Mus 127 originally was isolated was killed by an accident on February 16, 1944. At this point of time, 3½ months after it had been caught, numerous leptospirae were still present in the kidneys.

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VITAMIN E AND PROGESTERONE.

INFLUENCE OF α -TOCOPHEROL ACETATE ON THE EFFECT OF PROGESTERONE BY INTRAUTERINE AND SUBCUTANEOUS APPLICATION IN INFANTILE RABBITS.

By *Kjeld Halvorsen.*

(Received for publication April 19th 1944.)

For several years, vitamin E has been employed in the therapy, especially in cases of habitual abortion, given alone or together with corpus luteum preparations. The successful manufacturing of pure synthetic vitamin E preparations (»Ephynal« = α -tocopherol acetate) has now made it practicable to carry out more exact studies on the relation of vitamin E to the sex hormones. In the last years, several works have been published, especially by German authors — *e. g.*, Winkler; Stähler and collaborators — who have taken up the problem concerning synergism between vitamin E and the production and action of the female sex hormones; and it has been discussed whether the effect is a direct potentialization of the mucosal effect of progesterone on the endometrium, or whether the effect might assert itself through the ovary or the pituitary.

Thus, Stähler & Kaiser (6) and Stähler & Pehl (5) reported in 1941 that in infantile female rats kept on a diet poor in vitamin E they were able by an addition of 3 mg. Ephynal daily by mouth from the commencement of the preliminary treatment to reduce the progesterone dose to 1/10 and yet obtain the same mucosal phase as found in rats which did not receive this addition. Here it is to be pointed out that the authors largely base their statement on the glycogen deposit in the endometrium as the test, because the morphological changes in rats are not particularly pronounced. In experiments on infantile rabbits, which were given an addition of 9 mg. Ephynal daily by mouth during the experimental period, they obtained a mucosal effect corresponding to one Clauberg unit with 0.5 mg. progesterone.

Stähler & Hopp (4) state that in adult castrated female rabbits with a preliminary treatment of 2.5 γ estradiol benzoate daily for 8 days they obtained a secretory phase in the endometrium by admini-

stration of 500 mg. and 1235 mg. Ephynal. They found that 2500 mg. Ephynal in its effect corresponds to 1 mg. progesterone.

In all these experiments, unfortunately, only very small animal materials were employed: 1—2 animals in each group, and only very few controls. This means a very marked limitation of the value of the works reported. The most interesting observation is that it has

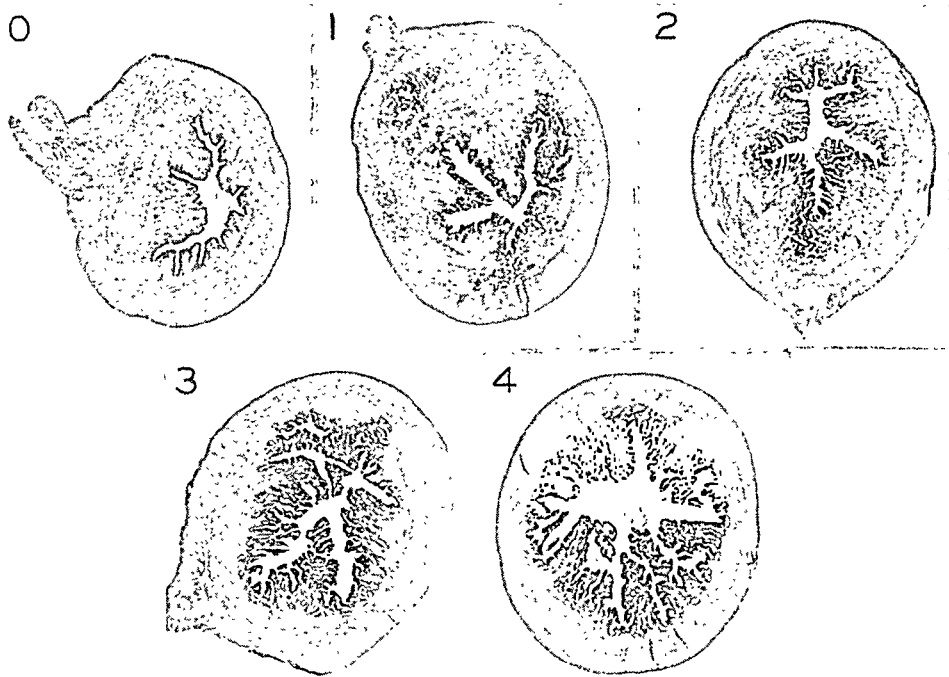


Fig. 1.
Reactions after McPhail.

been practicable at all to produce a secretory phase in the endometrium with the very large doses of Ephynal in castrated rabbits. In this connection, it is to be kept in mind that suprarenal cortical hormone has a certain progesterone effect, so that the striking effect obtained in the castrated rabbits may conceivably have been produced by way of the suprarenals.

Winkler (8, 9) states that on administration of 18 mg. Ephynal daily throughout a normal cycle in women, he found the secretory phase of the endometrium prolonged, with a quantitative increase in the pregnanediol output. Patients with threatening abortion for 2—3 months showed subnormal values for pregnanediol output, but on administration of 36 mg. Ephynal daily from the end of the second month, the pregnanediol output was doubled.

Winkler (7) likewise states that by administration of vitamin E to women he has been able to produce an increase in the urinary excretion of estrin and gonadotrophin. Unfortunately, he has failed to

give any particular account of his technique employed for these measurements; and while biological titrations normally show a total estrin excretion varying between 20 and 200 M. U., Winkler records in one place, for instance, and increase from 1000 γ to 1700 γ per day (1 γ = about 3 M. U.). So we shall not be able to accept these results until we are given a thorough account of the technique employed. For the same reason we may accept the report about a rise in the gonadotrophin excretion from 13 to 15 M. U. only with reservation. An experimental study by Winkler & Cyrenius (10) on the potentializing effect of vitamin E on the ovarian function in rats is inadequate as to the control material and the plotting of the estrin curves.

In contrast to Winkler, Roemer (2) obtained no particular results from vitamin E treatment for menstrual disturbances and sterility, and he was unable to confirm any synergism between vitamin E and follicular hormone.

Bomskov & von Kaulla (1) think it is just vitamin E deficiency which might bring about an increased effect of progesterone.

Varangot & Delor (3) examined the excretion of pregnanediol and steroid substances with the urine from 8 pregnant women before and after daily administration of 30 mg. α -tocopherol for 7 days without being able to demonstrate any definite difference.

During my experiments with intrauterine application of progesterone, in which I succeeded in demonstrating endometrial transformation with doses as small as about 0.01 γ progesterone, I had occasion also to try the effect of Ephynal administered in this way, which would reveal an eventual direct progesterone-like effect on the endometrium. Because of the negative results, these experiments have not been carried out on any large scale. With a view to the findings reported by Winkler and Stähler, I still think the publication of these experiments may be of some interest.

Technique.)*

Infantile female rabbits, weighing 800—900 g., belonging to a good strain, are given preliminary treatment with 150 I. U. of estrin in aqueous solution, subcutaneously, daily for 6 days. After a pause of 1 day, operation under ether anesthesia: Through a small incision in the abdomen, a uterine loop is laid free, 16—18 mm. of it being isolated by means of 2 ligatures. Prior to the tightening of the last ligature, a cannula is introduced through the uterine wall, outside

*) The Ephynal here employed was kindly placed at our disposal by F. Hoffmann-La Roche & Co., while we were generously supplied with progesterone by «Ciba».

the ligature, into the lumen of the uterus; and after tightening of the ligature 0.05 cc. of an oily solution containing the substance under analysis is injected into the uterine lumen.

After 72 hours, the animal is killed. The uterine segment is removed, and, after cautious evacuation of the oil, fixed in Bouin's fluid. The segment is divided into 4 parts, which all are embedded in the same paraffin block, so that a section of the block gives an average picture of the effect from the treatment throughout the segment. Staining with hematoxylin-picroeosin; and reading of the result after McPhail's scale (see the picture). The average reaction for each group of animals is calculated and recorded in a coordinate system (see the curve).

Experiment I.

46 rabbits. Preliminary treatment with 150 I. U. of estrin \times 6. Intrauterine application of 0.05 cc. of arachis oil, containing respectively 25 γ , 2.5 γ , 0.25 γ , 0.025 γ , and 0.0025 γ progesterone.

Prg. 25 γ		Prg. 2.5 γ		Prg. 0.25 γ	
Rabb. No.	Reaction	Rabb. No.	Reaction	Rabb. No.	Reaction
1	4	9	3	18	2
2	3-4	10	3	19	2
3	3	11	4	20	2
4	3-4	12	3	21	1
5	4	13	1-2	22	2
6	3	14	3	23	1-2
7	4	15	4	24	1
8	3-4	16	1	25	2
		17	2	26	1
Average reaction		Average reaction		Average reaction	
3,6		2,7		1,6	

Prg. 0.025 γ		Prg. 0.0025 γ	
Rabb. No.	Reaction	Rabb. No.	Reaction
27	2	37	0
28	1	38	0
29	3	39	1
30	1	40	0-1
31	0-1	41	0
32	1	42	0
33	0	43	0
34	0-1	44	0
35	1	45	1
36	1	46	0-1
Average reaction		Average reaction	
1,1		0,3	

Experiment II.

35 rabbits. Preliminary treatment with 150 I. U. of estrin \times 6. Then half of the animals are treated with subcutaneous injection of 10 mg. Ephynal

in aqueous solution $\times 5$ simultaneously with the injections of progesterone, the total dose of which is distributed over 5 daily subcutaneous oil injections.

Prg. 1 mg		Prg. $\frac{1}{2}$ mg		Prg. $\frac{1}{4}$ mg	
Rabb. No.	Reaction	Rabb. No.	Reaction	Rabb. No.	Reaction
47	3—4	54	3	50	0
48	3—4	55	2	60	0
49	4	56	2—3	61	3—4
50	3—4	57	3—4	62	0
51	3—4	58	2—3	63	1
52	3—4			64	2
53	3—4				
Average reaction		Average reaction		Average reaction	
3,6		2,7		1,1	

Prg. 1 mg + 5 \times 10 mg Eph.		Prg. $\frac{1}{2}$ mg + 5 \times 10 mg Eph.		Prg. $\frac{1}{4}$ mg + 5 \times 10 mg Eph.	
Rabb. No.	Reaction	Rabb. No.	Reaction	Rabb. No.	Reaction
65	4	70	3	75	0—1
66	3—4	71	1—2	76	2
67	3—4	72	3—4	77	1—2
68	3—4	73	2	78	1
69	3—4	74	2	79	2
				80	3
				81	0—1
Average reaction		Average reaction		Average reaction	
3,5		2,4		1,5	

The results from Exp. I and II are presented graphically in Fig. 2. As will be noticed, Ephynal gave no potentialization of the progesterone effect in Exp. II.

Experiment III.

10 rabbits. Preliminary treatment with 150 I. U. of estrin $\times 6$. On operation, intrauterine application of 1500 γ α -tocopherol acetate (Ephynal) dissolved in oil in half of the animals; in the remaining animals, intrauterine application of 150 γ in 0.05 cc. of oil.

In either group, no animal gave any positive reaction.

Experiment IV.

10 rabbits. From the commencement of the preliminary treatment, daily subcutaneous injection of 3 mg. Ephynal in oil. (The optimal dose for man is stated by Winkler to be 30 mg.) 5 animals, are given by intracuterine application, the mean-reaction dose of progesterone; 5 are given by subcutaneous application, the mean-reaction dose.

5 rabbits, 2.5 γ progest. i. ut.	average reaction: 2.75
5 " 500 γ " subcut. "	" 3.1.

On comparison with the standard curve, it will be noticed that no potentializing effect after preliminary treatment with vitamin E can be demonstrated in this experiment.

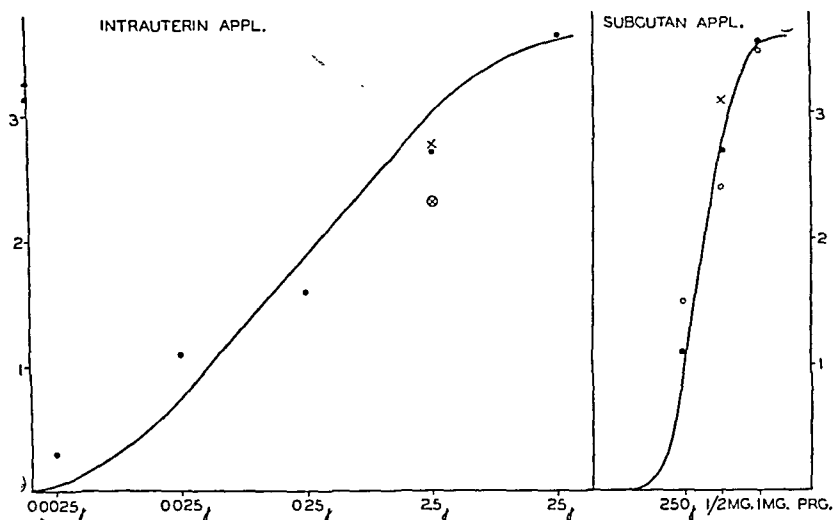


Fig. 2.

Ordinate: Reaction after McPhail (see Fig. 1.)

Abscissa: Dose of progesterone.

●: Average for *control groups* in Exp. I and II.

○: Overage for treated groups in Exp. I, with addition of Ephylnal simultaneously with the application of progesterone.

×: Average for treated groups in Exp. IV, with addition of Ephylnal from the commencement of the preliminary treatment.

⊗: Average for the treated group in Exp. V, with intrauterine application of progesterone and Ephylnal in the same dose of oil.

Experiment V.

5 rabbits. Preliminary treatment as usual. On operation, intrauterine application of 2.5 progesterone + 750 Ephylnal dissolved in 0.05 cc. of oil. Average reaction: 2.3.

So in this experiment too, no potentializing effect from Ephylnal could be demonstrated.

Discussion.

All the individual results in Exp. I and II are recorded in order to convey an impression of the wide dispersion of the results in working with progesterone, both on subcutaneous and intrauterine application, even with uniform animals of a good strain.

It will be highly appropriate, then, urgently to stress the importance of caution in estimating the experimental results obtained with progesterone and insist upon really adequate animal materials.

Exp. III, in which the effect of Ephylnal was tested directly on the uterine mucosa, gave a negative result even with doses that are more than 1000 times greater than the amounts of progesterone giving strong reactions. Neither additional Ephylnal from the very start of

the preliminary treatment or given together with the progesterone application showed any potentializing effect (Exp. II and IV). Also the experiment with intrauterine application of a solution containing both the mean-reaction dose of progesterone and 750 γ Ephynal, turned out negative (Exp. V).

Unfortunately, I have not had occasion to try out the effect of very large amounts of Ephynal on adult castrated rabbits.

In the present experiments, then, α -tocopherol acetate has had no direct or indirect potentializing effect on the action of progesterone on the endometrium in infantile rabbits treated preliminarily with estrin.

Summary.

The purpose of these experiments has been in infantile, estrin-treated rabbits to show whether Ephynal (α -tocopherol acetate) has any progesterone effect on the endometrium or potentializes the effect of progesterone on the endometrium. A total of 106 infantile rabbits were employed, each weighing 800—900 g., all receiving a preliminary treatment with 150 I. U. of estrin daily for 6 days. 46 rabbits were employed for a control curve, with intrauterine application respectively of 25 γ , 2.5 γ , 0.25 γ , 0.025 γ , and 0.0025 γ progesterone.

18 rabbits were employed for a control curve with subcutaneous application of 1 mg., $\frac{1}{2}$ mg., and $\frac{1}{4}$ mg. progesterone.

10 rabbits were given a direct intrauterine application of Ephynal.

10 rabbits received an addition of 3 mg. Ephynal daily from the commencement of the preliminary treatment, and mean-reaction doses of progesterone, by subcutaneous and intrauterine administration.

17 rabbits received an addition of 10 mg. Ephynal daily for 5 days simultaneously with subcutaneous application of progesterone.

5 rabbits were given a uterine injection of a solution containing both progesterone and Ephynal.

In no instance could any progesterone effect, direct or indirect, from Ephynal be demonstrated.

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ON THE FORMATION OF BACTERIAL ANTIBODIES IN TISSUE CULTURES

A REVIEW AND AN EXPERIMENT

By *E. Selmar*.

(Received for publication April 19th 1944).

Since the paper published by *Pfeiffer & Marx*¹) in 1898 and, especially, in recent years, numerous experiments appear to show a cellular origin of the bacterial antibodies (e. g., *Sabin*²), 1938; *Landsteiner & Parker*³), 1940; *Bjorneboe & Gormsen*⁴), 1941).

Sabin thinks she has been able by injection of a red protein antigen into rats to demonstrate the presence of an antigen-reticuloendothelial system-antibody mechanism. She found that the injected red granules through phagocytosis were taken up by reticuloendothelial cells, and she observed that these granules were losing their color and finally became invisible under the microscope. At the same time there was a detachment of exoplasm from the cells concerned (which now are designated as clasmatocytes and are identical with macrophages) and antibody appeared in the serum. Besides the colored protein, also the leucocytes underwent phagocytosis after they had transported the dye granules out into the reticuloendothelial system.

Considering that antibody now has been shown to be chemically identical with serum globulin (*Heidelberger & Kabat*^{3a}), 1934), it is rather interesting that *Landsteiner & Parker* in experiments in vitro have shown that cells are able to form serum globulin. The latter cultivated fibroblasts from chick embryos in 35 weekly passages in rabbit plasma and tissue juice from rabbit fetuses. After this, the tissue extract was found to precipitate on addition of chicken serum antibody, although presence of original chicken plasma protein should be excluded. The reaction was specific and vital. According to *Parker*, the above-mentioned fibroblasts in the reticuloendothelial system may be transformed reversibly to macrophages.

Through massive immunization of rabbits with up to 8 pneumococcus types simultaneously, *Bjorneboe & Gormsen* made practically

the entire amount of serum globulin appear as antibody at the same time as there was a marked increase in the number of plasma cells — especially in the spleen, which increased in weight. The authors think that plasma cells especially are able to form only antibody globulin, not serum globulin.

It is the writer's aim in this paper to show what experiments in vitro have contributed to elucidation of the question: cells/bacterial antibodies. Before mentioning my own experiment, however, it will be appropriate to review the experiments reported by other investigators.

Previous Experiments.

In 1912, the year after he had founded the modern technique of tissue culture, *Alexis Carrel* — in collaboration with *Ingebrigtsen* — showed that tissue cultivated in vitro was able to form antibody. These investigators⁵⁾ cultivated, in Gabritschewski dishes, guinea-pig bone marrow and lymph node in guinea-pig plasma with addition of goat corpuscles. On the 3' day the leucocytes were seen to have become active and capable of phagocytosis; on the 4'—5' day it was practicable in tissue extract from the contents of the dishes to demonstrate the presence of a specific complement-binding hemolysin against goat corpuscles. No information was given about the titer.

Since then, numerous experiments have been made on the formation of various antibodies in vitro⁶⁾, and efforts have been made to find which tissues are particularly good antibody producers. An experiment aimed to show the importance of the reticuloendothelial system in this respect was reported by *Meyer & Loewenthal*⁷⁾, who employed »milk spots« from rat omentum, which are claimed through a number of transfers to be able to yield reticuloendothelial cells in pure culture. These authors show that such a pure culture, grown on a slide, on addition of typhoid bacilli is able to form agglutinin for these bacteria. This is a non-pure experiment in vitro, however, as, for the sake of a sure intimate contact between the antigen and the tissue, the experiment was commenced by intravenous injection of the bacteria before the rat was killed. (This is the meaning of the term »non-pure experiment in vitro« employed in the following.) *Meyer & Loewenthal* obtained a maximum titer of 320. No control tests are reported.

Another »pure« tissue which has been examined for the faculty of antibody formation is corneal tissue from a 14-day old chicken embryo, which *Poleff*⁸⁾ (1928) let stay for 2 hours in a suspension of killed paratyphoid A bacilli. Then the tissue fragments were cultivated for 4—5 days in Carrel flasks. Extracts from the contents of these flasks were found to contain agglutinin with a maximum titer of 100 (200). Control titer: 0.

In a pure experiment in vitro in fluid medium, *Kimura*⁹⁾ (1926), employing rabbit spleen, found agglutinin for colon bacilli. Maximum titer: 40. Control titer: 0.

In a similar way, *Sato*¹⁰⁾ (1931) has demonstrated agglutinin formation for chicken cholera bacilli in cultures of chicken spleen, with a maximum titer of 16. Control titer: 4. In non-pure experiments in vitro *Sato* has further demonstrated a formation of hemolysin (rat spleen + goat corpuscles; maximum titer 16 control titer 8) and precipitin (rat spleen + horse serum maximum titer 5 control titer 0).

In non-pure experiments in vitro, *Komatsu*¹¹⁾ (1930, 1931) has in fluid medium demonstrated the formation of agglutinin (rabbit spleen and bone

marrow + typhoid bacilli) and bactericidin (rabbit spleen + bone marrow + lung with addition of typhoid bacilli) with maximum titers of 8 and 10, respectively, when the control titers are »shifted« to 0. No antibody production was found in the lung tissue, however.

In a pure experiment *in vitro*, *Schilff*¹²) (1926) thought he was able to demonstrate the formation of vibriolysin for El Tor by cultivation of fragments of spleen together with this cholera bacillus. But, while *Komatsu* employed an objective measure for the bactericidin formation (inhibition of the growth of colonies on agar plates), *Schilff* adopted a more subjective microscopic estimation of the degree of vibriolysis in samples of the peritoneal fluid from Pfeiffer's experiment.

An even less reliable foundation for any definite conclusion is met with in older experiments with inconstant results¹³) or with employment of a dubious cultural technique^{14 15}).

The following investigators have been unable to demonstrate any antibody formation *in vitro*.

*Werthemann*¹⁶) (1925) employed rat spleen and sheep corpuscles or typhoid bacilli. *Bloom*¹⁷) (1927) used rat lung and pigeon corpuscles. *Salle & McOmie*¹⁸) (1937) employed chicken embryo tissue together with spleen and lung from rabbit and guinea-pig, with addition of heterologous sera or blood corpuscles. Finally, *Parker*¹⁹) (1937) used rabbit spleen and guinea-pig corpuscles under employment of a particular technique, which was claimed to enable the tissue fragments to survive functionally without visible growth of the constituent elements. In particular, *Parker* was unable to reproduce the result reported by Meyer & Loewenthal.

Writer's Own Experiment, performed on August 9—16, 1943.

The aim was to examine the fluid medium for the possible presence of agglutinin for *Bac. abortus* Bang after cultivation for various numbers of days. Spleen and heart tissue from 19-day-old chicken embryos were grown first for 24 hours on solid medium, bathed at 39° in a suspension of killed *abortus* bacilli (optic concentration corresponding to 10¹⁰ colon bacilli/cc., heated twice to 65°, with addition of 0.01 % methiolate). Then this contact between antigen and tissue was interrupted by repeated washing with Tyrode's fluid. The cultivation was carried out in Carrel flasks containing:

Solid medium:	27	Tyrode fluid.	
	15	»	chicken plasma.
	3	»	chicken embryonic extract.
Fluid medium:	7	Tyrode fluid.	
	5	»	chicken plasma.
	3	»	chicken embryonic extract.

In these flasks were cultivated: 10 pieces of heart tissue + 4 pieces of spleen (each less than 1 mm. for all dimensions).

The experiment was carried out with 2 flasks, and the fluid medium was removed herefrom daily and replaced with fluid medium from accessory flasks, in which the fluid medium was changed every 2 days. Thus it was practicable, without interfering too much with the metabolism of the experimental tissues, to perform a Vidal test

on the 1', 2', 3', 4' and 7' day, with examination for the titers $1\frac{1}{4}$, $2\frac{1}{2}$, 5, 10, 20 and 40 after incubation of the Vidal tubes for 6 hours at 50°. *The result was entirely negative.*

Abortus bacilli Bang were employed because they are particularly willing to give agglutination. This antigen-antibody reaction was preferred because it leaves but little to discuss.

Prior to the experiment, I had made sure that this race of chicken was able to form agglutinin for *Bac. abortus* Bang by intravenous injection of 2—1—2 cc. of the bacterial suspension employed in the experiment — into a cock, every other day. After 14 days, the serum titer was found to be 400.

At the conclusion of the experiment — in order to make sure that the tissue remained vital throughout the experiment — random samples were taken of the experimental and the accessory tissues for further cultivation. Slide cultures of these tissue samples showed fine growth (comparison of outlines drawn by means of a projection apparatus at intervals of 2 days).

Several investigators permit the contact between antigen and tissue throughout the experiment. I did not find this advisable, as it seemed conceivable that the antigen in this way might fix the antibody gradually as it was formed. In this way a positive Vidal might turn out negative. Still, also investigators who have reported a positive result appear to have failed to take this point into account.

Sato — whose experiment is the only one in the above survey with which my own experiment is comparable — obtained agglutination in the »2' tube« (if we »shift« the control titer to 0 and imagine a series of dilutions 1 — 2 — 4 — 8 etc.). I was unable even to obtain this modest result, in spite of a protracted and »particularly vital« contact between the antigen and tissue.

Conclusion.

In pure experiments *in vitro*, some investigators have found relatively small antibody titers, while others (and more recent) have been unable to demonstrate any antibody production. The outcome was negative also in the present experiment on agglutinin formation against *Bac. abortus* Bang by tissue cultures of heart and spleen of chicken embryos.

Summary.

After mention of some experiments that lend support to the assumption of a cellular origin of the bacterial antibodies, a survey is given of the results reported in previous experiments with tissue cultures *in vitro* aimed to elucidate the question about the relation of the bacterial antibodies to the cells.

Finally, a report is given of the author's own experiment with employment of cultures of heart and spleen from chicken embryos together with Bac. abortus Bang. No agglutinin formation could be demonstrated.

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DANSK PATOLOGFORENINGS 8. MØDE 20/1 1943 I KØBENHAVN.

The eight Meeting of the Danish Pathological Society, January 20, 1943.

Huitième séance des pathologistes danois, le 20 janvier 1943.

Achte dänische Pathologentagung am 20 Januar 1943.

Johns. Meyer: *On the Significance of Traumatic Injury to the Localization of Inflammatory Processes, especially Tuberculosis.*

To be published in in extenso in this journal.

Discussion.

Harald Gormsen: On microscopic examination of macroscopically normal bone-marrow from the femur and sternum of patients who died of pulmonary tuberculosis but had no miliary tuberculosis, in 8 out of 20 cases I have found rather large typical tuberculous foci which most often contained rather many tubercle bacilli.

On going through corresponding autopsy specimens of bone-marrow from about 300 patients who died of different non-tuberculous diseases, I have found an epithelioid cell granuloma only in one case — a man, aged 62, suffering from cancer of the stomach and without any sign of recent or old tuberculosis (no tuberculin test performed) — and this process most resembled an old tuberculous focus, but no tubercle bacilli could be demonstrated in the sections.

In sections of sternal punctates on 31 patients with different forms of tuberculosis I did not find any tuberculous focus in any instance, whereas epithelioid cell granulomas in sternal punctates from patients with Boeck's disease are found rather frequently (I have observed such granulomas in 5 out of 16 patients). It may be that the first-mentioned tuberculous foci in the bone-marrow of patients who died of pulmonary tuberculosis merely result from a terminal dissemination of tubercle bacilli. But it is also possible that these foci represent the latent tuberculous bone-marrow foci Dr. Meyer is looking for, and which undoubtedly will play a role in the pathogenesis of bone tuberculosis, especially bone tuberculosis on traumatic basis.

Johns. Holm: No abstract received.

K. A. Jensen: " " "

Hans Thomsen: I have occupied myself a good deal with the ques-

tion under discussion — not only as far as tuberculosis is concerned but also with regard to purulent osteomyelitis. I should like first to object to what I consider a mistaken analogy presented by Dr. Meyer in the last part of his paper, namely: that the favorable effect from immobilization could be taken to support the view that a traumatic injury may aggravate a preexisting tuberculosis. The slight daily »traumatic strain« which a tuberculous extremity is exposed to is indeed of a character quite different from the effect of a single well-defined traumatic injury. According to my own experiences from about 300 operations in a tuberculosis-infected field, no real spreading of the tuberculosis is ever seen even though these traumata — for instance in joint resections — are considerably more extensive than the highly variable and often indefinable injuries which the patients most frequently give as the cause of the aggravation of the tubercle foci.

As to the question whether a traumatic injury may favor the localization of an infection to the bone-marrow, I have to maintain that I do not understand what pathologic-anatomical changes should occur in the bone in the rather insignificant traumatic injuries which usually are given as the cause of the lesion. — On the whole, I wish to warn emphatically against relying on the anamnestic data in the case records unless the patient has been questioned critically with a view to this problem. It has been my experience again and again that patients with tuberculosis of tarsal bones or the knee-joint have insisted that they »missed their footing«, and then when I questioned them about the details of this accident they would hesitate and finally say »Oh, yes, I suddenly had some pain in my foot so I must have missed my step.«

As to the interesting roentgenogram presented by Dr. Meyer of a fracture of the spinal column, which was followed by the development of a tuberculous spondylitis, I think it really is not to be wondered that we will be able once in a great while to ascertain the coincidence of a fracture of spongy bone and development of a tuberculous process. Besides, as Dr. Meyer emphasized himself, one case alone is no convincing evidence.

I subscribe to the view advanced by Professor K. A. Jensen, that experimental studies are indispensable to the solution of this question, and I hope the problem will be taken up soon. But naturally, I shall not commit myself to say whether the future investigator will arrive at the standpoint suggested by Liniger when he says that we will have to »make a clean sweep of all antiquated theories concerning the traumatic tuberculosis and show clearly that osteomyelitis as well as bone tuberculosis are infectious general lesions which without any external cause localize to certain typical regions, not particularly to regions hit by traumatic injury« — and I subscribe to the same view.

J. Engblom-Holm. No abstract received.

DANSK PATOLOGFORENINGS 9. MØDE 24/3 1943 I KØBENHAVN.

The ninth Meeting of the Danish Pathological Society 24 March 1943.

Neuvième séance des pathologistes danois, le 24 mars 1943.

Neunte dänische Pathologentagung am 24 März 1943.

Vitamin- und Antivitaminwirkung auf Hefe

von

Niels Nielsen und Gordon Johansen,

Carlsberg Laboratorium.

Während über die Substanzen, die gegenüber p-Aminobenzoesäure als Antivitamin oder Antiwuchsstoff wirken, eine Reihe von Arbeiten erschienen ist, liegen bis jetzt fast keine Untersuchungen über die Substanzen vor, die für andere Wuchsstoffe als Antiwuchsstoff wirken, obwohl man von vornherein vermuten darf, dass derartige Untersuchungen von grossem Interesse sein müssten. Das ist besonders hinsichtlich der Antiwuchsstoffe der Pantothenensäure anzunehmen, und zwar wegen der grossen und allgemeinen Bedeutung dieses Wuchsstoffes für die Mikroorganismen. Kuhn, Wieland und Möller haben nachgewiesen, dass Sulfopantothenensäure, d. h. eine Pantothenensäure, die anstatt β -Alanin Taurin enthält, auf Milchsäurebakterien als Antiwuchsstoff gegenüber der eigentlichen Pantothenensäure wirkt; da letztere für das Wachstum dieser Bakterien notwendig ist, kann man ihr Wachstum durch Zusatz genügender Mengen von Sulfopantothenensäure verhindern.

Das Wachstum des im Carlsberg Laboratorium benutzten Hefestammes wird nicht nur durch Pantothenensäure, sondern auch durch β -Alanin gefördert, offenbar weil die Hefe selbst vermag, Pantothenensäure aus β -Alanin aufzubauen. Nach der von Kuhn und seinen Mitarbeitern festgestellten Wirkung der Sulfopantothenensäure auf Milchsäurebakterien war zu erwarten, dass Taurin gegenüber β -Alanin als Wuchsstoff wirkt. Unsere Versuche ergaben indessen, dass dies nicht der Fall ist. Taurin wirkt auf Hefe weder als Wuchsstoff noch als Antiwuchsstoff. Dagegen zeigten verschiedene Derivate des β -Alanins eine kräftige Antiwuchsstoff-Wirkung. Das gilt vor allem für β -Aminobuttersäure, die die Wuchsstoffwirkung des β -Alanins völlig aufheben kann. Diese β -Alanin-Derivate haben selbst keine Wuchsstoffwirkung, aber ebenso wenig hemmen sie das Wachstum der Hefe, solange kein β -Alanin zugesetzt wird (Tabelle 1, 1. Spalte); daraus geht hervor, dass die durch sie bewirkte Hemmung bei Anwesenheit von β -Alanin eine typische Antiwuchsstoffwirkung gegenüber diesem Wuchsstoff ist.

Gegenüber der Pantothenensäure wirken weder β -Aminobuttersäure noch andere Derivate des β -Alanins als Antiwuchsstoffe. Da die Wuchsstoffwirkung des β -Alanins vermutlich auf seiner Umwandlung zu Pantothenensäure beruht, so ist anzunehmen, dass die Antiwuchsstoffwirkung von β -Aminobuttersäure, Isoserin und Diaminopropionsäure darin besteht, dass sie bei Bildung von Pantothenensäure das β -Alanin verdrängen, sodass unwirksame Pantothenensäure-Derivate entstehen. Wir haben also hier einen neuen Typus der Antiwuchsstoffwirkung. Bei der Hefe lassen sich offenbar zwei Stufen der Antiwuchsstoffwirkung unterscheiden. Man kann die Entstehung des eigentlichen Wuchsstoffes, der Pantothenensäure, mit Hilfe von β -Aminobuttersäure, Isoserin oder Diaminopropionsäure verhindern, man kann aber sicher auch später die Wirkung der Pantothenensäure durch Zusatz von Pantothenensäure-Derivate aufheben. Die letztere Möglichkeit ist noch nicht abschliessend geprüft.

Tabelle 1.

Nährlösung mit 0,20 γ Biotin, 50 γ Aneurin und 200 mg Glutaminsäure.

Zugabe je 50 ccm	mg Hefe-Trockensubstanz je 50 ccm		
	Ohne Zugabe	mit 1 γ β -Alanin je 50 ccm	mit 0,75 γ d,1-Na- Pantothenat je 50 ccm
10 mg β -Aminobuttersäure	4,8	5,2	21,0
3 " "	4,7	7,1	20,6
1 " "	4,9	16,2	20,9
0 (Kontrolle)	5,0	20,0	21,3
10 mg Isorin	5,3	10,1	21,5
3 " "	5,0	12,3	21,2
1 " "	5,1	18,7	20,9
0 (Kontrolle)	4,8	21,2	21,1
10 mg Diaminopropionsäure	4,7	9,8	20,0
3 " "	4,9	10,9	19,6
1 " "	5,1	17,8	19,9
0 (Kontrolle)	5,2	19,8	20,1

Keine Diskussion.

Inga Scheibel: *Experimental Studies on Active Immunization with Combined Diphtheria and Tetanus Vaccine.**)

Guinea-pigs immunized with combined diphtheria-tetanus vaccine showed lower tetanus antitoxin titer than did guinea-pigs immunized with tetanus vaccine alone. The diphtheria-antitoxin titer, on the other hand, was the same in the two series of animals. Statistical calculations showed that the difference between the tetanus titers was significant, and hence we have to subscribe to the theory maintained by Michaelis and other investigators. about »Konkurrenz der Antigene«.

In no instance have we, like Ramon and collaborators, been able to demonstrate an increased antibody production resulting from vaccination with combined vaccine. The antigen competition appears in part to be independent of the dosage.

Considering the relatively abundant antibody formation for both antigens that took place in spite of the antigen competition, we think the method will be serviceable for human vaccination. For this purpose we have prepared a vaccine which, in the same dosage and with the same number of injections, as are now employed in diphtheria vaccination, should be able at the same time to give immunity against diphtheria and tetanus. Experiments on human subjects for additional documentation of this are now going on.

Discussion.

Axel Perdrup: A mixture of diphtheria and tetanus anatoxin gives no decrease in the diphtheria immunity obtained and only a slight diminution in the tetanus immunity as compared to the immunity obtained by employment of separate vaccines. So the person who is vaccinated in this way acquires his immunity against tetanus without any discomfort at the same time as he is vaccinated against diphtheria.

In Denmark the serum prophylaxis against tetanus is carried out to such

*) Will later be published in extenso.

an extent that we cannot expect to obtain additional results in this way. The only means of lowering the tetanus morbidity further is vaccination of as many people as possible. To me, then, it seems highly important that the employment of this mixed vaccine be adopted for general practice as soon as possible.

Additional discussion by E. Juel Henningsen, J. Ørskov and Johs. Ipsen.

F. Kauffmann: »Über pathogene Coli-Typen. Ein Beitrag zur Ätiologie der Appendicitis und Peritonitis.«

Es wurden 92 Coli-Kulturen kulturell und serologisch untersucht, und zwar 42 Stämme aus pathologischem Material (Urin, Eiter, Blut etc.) sowie 50 Stämme aus Faeces. Von allen 92 Kulturen wurden O-Seren und OL-Seren hergestellt, ausserdem von 20 verschiedenen Kulturen H-Seren.

Zur serologischen Typenbestimmung kamen in erster Linie die O-Antigene in Betracht, durch deren Analyse 58 verschiedene O-Gruppen aufgestellt wurden.

Die Gruppenhäufigkeit war bei Stämmen aus pathologischem Material und aus Faeces deutlich verschieden, da einige wenige Gruppen besonders häufig im pathologischen Material vorkamen, sodass sie im Besitze einer besonderen *Pathogenität* sein müssen. Diese Gruppen waren dadurch charakterisiert, dass sie aus verschiedenen *Typen* bestanden, die in einem hohen Prozentsatze der Fälle serologisch verschiedene *L-Antigene* enthielten und deshalb O-inagglutinabel waren.

Unter Berücksichtigung der O- und L-Antigene konnte ein *diagnostisches Antigeneschema* aufgestellt werden, in das jedoch nur solche Gruppen aufgenommen wurden, die wiederholt gefunden sind, oder in denen L-Antigene nachgewiesen wurden.

Haemolytische Coli-Stämme kamen besonders häufig in einigen wenigen, pathogenen O-Gruppen vor; doch waren spezielle Typen derselben O-Gruppen sowie andere pathogene O-Gruppen nicht haemolytisch.

Aus den vorliegenden Untersuchungen wird geschlossen, dass es *bestimmte, serologisch definierte Coli-Typen* gibt, die eine grössere Menschenpathogenität als gewöhnliche Faeces-Stämme besitzen. Besonders eindrucksvoll trat dieser Unterschied bei der Untersuchung von Kulturen aus *Peritonitis-Fällen* (nach Appendicitis) in Erscheinung.

Es wird die Arbeitshypothese aufgestellt, dass *pathogene Coli-Typen* als Erreger von *Appendicitis* und *Peritonitis* an erster Stelle stehen, während grampositive Kokken und Anaerobier seltener als Erreger auftreten und meist als Begleitbakterien aufzufassen sind.

Da als Haupterreger von Appendicitis und Peritonitis bestimmte, pathogene Coli-Typen in Betracht kommen, so ist die Möglichkeit gegeben, das *Peritonitis-Serum*, unter Berücksichtigung der L-Antigene, erheblich zu verbessern.

Bei Coli-Cystitis- und Pyelitis-Fällen konnten besondere, pathogene Coli-Typen in etwa der Hälfte der Fälle festgestellt werden, sodass damit zu rechnen ist, dass auch gewöhnliche Faeces-Kulturen unter bestimmten Bedingungen Infektionen verursachen.

Die bisherige Einteilung der Coli-Gruppe auf Grund *kultureller* Merkmale in *coli communis*, *communior*, *acidi lactici* u. s. w. ist verfehlt und durch die *serologische* Einteilung zu ersetzen.

Dr. M. Kristensen: Dr. Kauffmann's Bemerkung, dass die vorgelegten Untersuchungen ohne Bedeutung für die bakteriologische Diagnosestation sind, soll wohl nur für die nächste Zukunft gelten. Ich hoffe, dass die Unterscheidungs-Merkmale zwischen pathogenen und apathogenen Colibazillen

früher oder später eine unmittelbare, praktische Bedeutung erhalten werden, z. B. zur Bestimmung, ob Colibazillen, die in Nahrungsmitteln gefunden werden, als Ursache von Darminfektionen in Betracht kommen können.

Mit Rücksicht auf das Verhältnis zwischen L-Antigen und Pathogenität könnte man vielleicht fragen, ob es undenkbar wäre, dass das L-Antigen bei einem gegebenen Colistamme hervortrat oder vermehrt wurde, wenn er seine pathogene Wirkung entfaltete, sodass also dieses Antigen zum Teil sekundär im Verhältnis zur Pathogenität sein könnte?

Dr. Kauffmann erklärte die vergärungsmässig definierte Gruppeneinteilung der Colibazillen für völlig verfehlt. Dieses ist sicher richtig, wenn man sich nur für die Frage der pathogenen Eigenschaften interessiert, aber es muss wohl nicht all' zu absolut genommen werden. Die Einteilung der Coligruppe in weiterem Sinne mit Hilfe von Vergärungs- und anderen biochemischen Reaktionen hat doch wohl eine gewisse Beziehung zum Vorkommen bei warmblütigen Tieren, kaltblütigen Tieren und in der freien Natur; vielleicht kann man auch von einer rein theoretischen Systematik sprechen, und in solchem Falle kann man die Vergärungsverhältnisse sicher nicht ausser Betracht lassen.

Further discussion by J. Ørskov, H. Ewertsen, E. Juel Henningesen, Johs. Ipsen, A. Søeborg Ohlsen, A. Perdrup, V. Friedenreich and P. Holm.

Dr. F. Kauffmann: Ob meine Schlussbemerkung, dass die Typenbestimmung innerhalb der Coligruppe in erster Linie rein wissenschaftliches Interesse hat und für die bakteriologische Praxis der Diagnosestationen nicht in Betracht kommt, zu Recht besteht, muss die weitere Entwicklung zeigen. Im heutigen Anfangsstadium der Coli-Serologie lässt sich eine sichere Entscheidung nicht fällen.

Ich habe selbst daran gedacht, dass die L-Antigene eventuell erst dann hervortraten oder vermehrt wurden, wenn die Bakterien Gelegenheit erhielten, ihre pathogenen Eigenschaften zu entfalten. Ich habe deshalb wiederholt versucht, das fehlende L-Antigen bei mehreren Coli-Stämmen hervorzurufen, indem ich die Kulturen Mäusen i. p. injizierte und weiterhin durch Mäuse passieren liess, ohne dass aber eine Änderung der Antigenstruktur eintrat. Wie schon früher erwähnt, ist ein L-Formenwechsel bei Coli-Bakterien selten, da die Stämme ihre serologischen Eigenschaften zäh festhalten. Ich glaube daher, dass L-Antigene bei bestimmten Coli-Typen von Anfang an vorhanden sind, zumal solche Kulturen das L-Antigen gut entwickelt haben, selbst wenn sie aus normalen Faecesproben isoliert werden.

Betreffs der kulturellen Untersuchungsmethoden bin ich mit Dr. Kristensen derselben Meinung, da ich in meinem Vortrage darauf hinwies, dass gewisse kulturelle Untersuchungen, wie das Verhalten auf Ammoncitratagar und in Gelatine von grosser Bedeutung sind, wenn man eine »Coli-Gruppe im engeren Sinne« von der grossen, heute noch nicht klar übersichtbaren Gruppe gramnegativer Bakterien abtrennen will. Für die Abgrenzung bestimmter Coli-Typen, die bei verschiedenen Erkrankungen, vor allem bei Peritonitis nach Appendicitis, überwiegend vorkommen, ist aber allein die serologische Methode das Entscheidende, wie es auch Dr. Kristensen betonte.

F. Kauffmann und Beate Perch: »Über das Vorkommen von *Proteus* X 19 in Dänemark. Ein Beitrag zur Theorie der Weil-Felix-Reaktion.« Aus den Faeces eines 3 Monate alten Knaben, der an akuter Gastroenteritis erkrankt war, wurde ein *Proteus*-Stamm isoliert, der mit dem von Weil und Felix gefundenen *Proteus* X 19-Stamme serologisch identisch war und mit »K P 21« bezeichnet wurde. Beide Kulturen waren auch in vergärungs-

mässiger Hinsicht identisch, unterschieden sich aber in ihrem Verhalten gegen Gelatine. Während der frisch isolierte Stamm KP 21 Gelatine nach 2 Tagen völlig verflüssigt hatte, war die mit dem X 19-Stamme beimpfte Gelatine nach 30 Tagen fest und zeigte nur ab 2.—4. Tage eine kraterförmige Verflüssigungszone an der Oberfläche. Dieser Befund beweist, dass die Züchtung des KP 21-Stammes *nicht* auf einer Laboratoriumsinfektion mit dem Stamme X 19 beruhen kann.

Von beiden Kulturen wurden O- und H-Immunseren hergestellt, die im gekreuzten Absorptionsversuche geprüft und als identisch befunden wurden. Hierdurch ist zum ersten Male der sichere Beweis geführt worden, dass *Proteus* X 19-Stämme auch bei Nicht-Fleckfieberkranken vorkommen. Derartige Stämme sind jedoch in Dänemark selten, da unter 300 *Proteus*-Kulturen nur 1 Stamm (KP 21) gefunden wurde.

Dieser Stamm eignete sich zur Weil-Felix-Reaktion in genau derselben Weise wie der X 19-Stamm. 3 Seren von Fleckfieberkranken agglutinierten beide Stämme gleich hoch. Das Serum des erkrankten Knaben agglutinierte beide *Proteus*-Stämme nicht.

Der Befund von *Proteus* X 19-Bakterien im fleckfieberfreien Milieu stützt die Ansicht, dass die Weil-Felix-Reaktion auf dem rein zufälligen Vorkommen ein und desselben Antigens bei 2 verschiedenen Mikroorganismen, *Proteus* X 19 und *Rickettsia* Prowazeki, beruht. Die Annahmen, dass zwischen diesen Keimen genetische Zusammenhänge bestehen, oder dass es sich um einen Fall von Paragglutination handelt, werden abgelehnt.

Diskussion: Dr. Ørskov. Keine Referat.

DANSK PATOLOGFORENINGS 10. MØDE 19/5 1943 I KØBENHAVN.

The tenth Meeting of the Danish Pathological Society, May 19, 1943.

Dixième séance des pathologistes danois, le 19 Mai 1943.

Zehnte dänische Pathologentagung am 19 Mai 1943.

H. Schledermann: *A case of malignant intracardiac heart tumor.*

After a brief survey of the tumors of the heart, a report is given of a case of intracardiac spindle-cell sarcoma with metastases to the lung and skull, disclosed as the cause of death in a woman, 36 years old.

The patient had been well until two months prior to admission when she had dyspnea and irritative cough.

After a couple of small hemoptyses at home, the patient was admitted to the Tuberculosis Dep. of the Frederiksberg Hospital. On the day after her admission she had a voluminous hemoptysis, under which she died.

Autopsy revealed the presence of a large tumor ($3\frac{1}{2} \times 5 \times 6$ cm) in the left atrium of the heart, and metastases in the left lung and in teca cranii.

Histologically the primary tumor and the metastases presented the structures of a spindle-cell sarcoma.

No discussion.

J. Bing and N. O. Christensen: *Continued Research into the Pathological Formation of Globulin.*

In the course of investigations into the occurrence of hyperglobulinaemia in a large number of animals, 34 cases of pronounced hyperglobulinaemia were found by means of the formol-gel reaction. They were mainly chronic infections, particularly strangles in horses and tuberculosis in cattle. In those cases where it was possible to obtain biopsy or autopsy we always found an abundance of plasma cells. One exception to this rule was a horse, in which an abscess membrane contained no accumulation of plasma cells, though there were some reticulo-endothelial cells, of which the protoplasm, like that of plasma cells, stained deeply with pyronin. (In this connection mention was made of some experiments, as yet incomplete, carried out in collaboration with Dr. Holter, which suggest that the staining of the protoplasm with pyronin is due to a high content of ribonucleic acid.)

Our investigations have thus confirmed that there is a connection between hyperglobulinaemia and plasma-cellular or other reticulo-endothelial reaction.

Discussion.

M. Bjørneboe: The hypothesis advanced by Bing and Plum about the plasma cells as producers of globulin appears to have a very wide applicability. Here it will be appropriate to call attention to the fact that the animal diseases mentioned by Drs. Bing and Christensen are infectious diseases, and that the demonstrated relation between the increase in globulin and the occurrence of plasma cells in these cases therefore lends support to the modification of Bing and Plum's hypothesis suggested by Bjørneboe and Gormsen, namely: that the plasma cells produce the antibodies, which are globulins.

J. Engelbreth-Holm: This paper is a valuable addition to the many Danish works showing the connection between the reticuloendothelial system, in this instance, plasma cells, and globulin — here antibody. It is to be kept in mind, however, that numerous morbid conditions are associated with very pronounced plasma cell infiltration without any accompanying increase in the globulin content of the serum.

Bing & Christensen: Naturally, it is possible that Bjørneboe and Gormsen are right in saying that it is only the antibody globulin that is formed from plasma cells. Nevertheless, at the present stage it is impossible to say if the hyperglobulinaemia in cases of carcinomatosis, uraemia, cirrhosis of the liver, leukaemia and myelomatosis is due to a formation of antibodies, and therefore we must for the present assume that plasma cells also form other globulins than antibodies.

To Professor Engelbreth-Holm we would say that we quite realize that there may be a considerable accumulation of plasma cells without hyperglobulinaemia; it is seen in about 20 per cent. of cases of myelomata.

E. Bruun & K. Hermann. *Neurotoxic Effect of Sulfonamides on Pigeons*. (Preliminary report.)

In previous papers the authors have reported a number of instances of neuritis and polyneuritis in man. It was found that uliron (sulfanilyl-dimethylsulfanilamide) and staphylamide (sulfamethylthiazole) were the cause of this phenomenon in most of the cases, while any of the sulfonamides employed at that time was able occasionally to give neuritis.

The authors therefore took up the task of investigating the neurotoxicity of the following substances on animals: sulfanilyl-dimethylsulfanilamide,

sulfamethylthiazole, sulfathiazole, sulfanilamide, sulfapyridine, sulfamethylthiodiazole and that is, 7 different sulfonamides.

The experiments were carried out on pigeons weighing about 400 g. The drug was given by means of a tube in a daily dose of 35 cg. \times 2. According to the previous experiences the authors thought that nerves under a strong strain and previously injured nerves are particularly liable to become the site of toxic neuritis and hence they have attempted also to elucidate the significance of this strain or past injury to the appearance of the neuritis by letting the animals walk in an electrical drum for half an hour daily, forcing them in this way to make use of their legs. Each drug was tried out on 8 pigeons, 5 of which were exercised in the drum, while the remaining 3 served as controls that were given sulfonamide but were not exercised in the drum. Each experiment lasted 3 weeks. With this equal dosage of all the sulfonamides employed, toxic neuritis was found to be produced in several cases by sulfanilamide, sulfamethylthiodiazole, sulfanilyl-dimethylsulfanilamide, and sulfapyridine. In addition, sulfanilamide and sulfapyridine had also a pronounced general toxic effect on the animals. On the other hand, sulfathiazole and sulfamethyldithiazole had a general toxic effect without giving neuritis. 2 (p- & ætansulfonsurt. Na-aminobenzol-sulfonamido) tiazol (alfasol) had no toxic effect, neither general nor neurotoxic. The animals which were exercised in the drum appeared to be more liable to neuritis than the controls.

With this uniform dosage, however, the concentration of the drug in the blood was found to vary very greatly — from about 1 mg. % (sulfanilyl-dimethylsulfanilamide) to about 100 mg. % (sulfamethylthiazole). On this account a new series of experiments was commenced aiming to keep the dosage at a »therapeutic level« i. e., with a concentration of the drug in the blood amounting to 6–12 mg. %. These experiments are not yet concluded, but it can be stated already that in order to obtain this »therapeutic« concentration of the drug in the blood with sulfanilamide, sulfamethylthiazole and sulfanilyl-dimethylsulfanilamide it is necessary to give respectively 3.5, 15 and 110 cg. of the substances concerned.

The experiments are being continued.

Erna Christensen: *Experimental Neuritis in Pigeons (Histology).*

Systematic histological studies on the effect of sulfonamides given in therapeutic doses were reported in 1940 by Bieter and collaborators, who found the effect to be least pronounced after administration of sulfonamide, and increasingly pronounced in succession after sulfapyridine, sulfamethylthiazole, sulfanilyl-dimethylsulfanilamide and sulfaphenylthiazole. The changes consisted in degeneration of nerve cells, medullary sheaths and axis cylinders, together with proliferative endarteritis of the cerebral blood vessels. Only after the last-mentioned drugs were the changes irreversible. The authors say nothing about the findings in the spinal ganglia, which would be of interest if the changes are to be compared with the findings in polyneuritis-polyradiculitis in man, where the most pronounced changes are found just in the spinal ganglia in the form of degeneration and round-cell infiltration.

Our histological studies apply to pigeons given sulfanilamide, sulfapyridine and sulfamethylthiodiazole in toxic doses and sulfanilamide and sulfamethylthiazole in therapeutic doses (blood concentration 6–12 mg. %).

Pronounced changes in the central nervous system were found in all the animals given toxic doses of the drug.

In the sulfathiazole-treated animals the brain presented swelling of the nerve cells and oedema, but endarteritis was seen neither here nor in the spinal cord. The spinal cord showed Nissl's severe cell disease and neurophagia, especially in the lumbar part of the cord. The same cellular

changes were seen in the spinal ganglia — focal round-cell infiltration and degeneration of medullary sheaths — which were seen in the radices. In addition, round-cell infiltration was seen in the ganglions and in the leptomeninges. Similar changes were found in the animals treated with sulfapyridine and sulfamethylthiodiazole; in particular, the latter group of animals presented a far more pronounced round-cell infiltration in the spinal cord.

Pigeons treated with therapeutic doses of sulfanilamide presented the same changes though far less pronounced. In the sulfamethylthiazole-treated animals many well-preserved nerve cells were seen in the spinal cord between the degenerated cells, and here the changes in the spinal ganglia and nerve roots were quite slight.

Knud O. Møller: Experiments like those here reported are associated with various difficulties. Owing to the widely differing absorption of the sulfonamides from the intestinal canal it is not practicable to correlate the dose of the various drugs with the effect obtained. The relation between the effect and the sulfonamide concentration of the blood can be recognized only when the concentration of the drug in the blood is kept fairly constant throughout the 24-hour period; and this means that in experiments of this kind at least 4—6 doses will have to be given in 24 hours — not, as here, a single dose. When, as here, the concentration of the remedy in the blood is estimated 4 hours after administration of a single dose, the results obtained will be difficult to utilize because the maximal concentration of the drug in the blood appears at different points for the various remedies.

Additional complications arise from the fact that the various sulfonamides differ greatly in their distribution in their organism. Some of them, e.g., (sulfanilamide) are distributed almost equally throughout the organism, whereas, for instance, sulfamethylthiodiazole (»Lucosil«) cannot be demonstrated at all in the cerebrospinal fluid. It seems reasonable to assume that the effect on the nervous tissues will depend on the sulfonamide concentration in these tissues, not on its concentration in the blood or plasma; and we are ignorant about the proportional distribution of the substance in plasma and nervous tissue. Furthermore, a smaller or greater part (up to about 80 %) of the sulfonamides in the plasma are adsorbed through the proteins and this part is not ultrafiltrable. So we have to admit, I think, that it will be difficult to estimate the neurotoxic properties of the sulfonamides by correlation of the effects obtained with a single determination of the concentration in the blood — let alone with a given dose.

The studies here presented are creditable, and they ought to be continued for in this way it may be possible to obtain a better understanding of the mechanism in the appearance of »neuritis« after administration of sulfonamides.

K. A. Jensen and K. H. Krabbe: No abstract received.

G. Vraa-Jensen: The material here presented is of considerable neuro-histological influence because it implies the possibilities of experimental investigation of the problems in connection with deterioration of the medullary sheaths. It will undoubtedly be of additional value if these studies were supplemented by polarization microscopy.

M. Faber: In the discussion of the studies here presented it has been taken as a matter of course that the neuritic changes would be directly attributable to the given sulfonamides. Several conditions might suggest, however, that the matter was not quite so simple as that. Quite similar neuritic

phenomena are observed in idiopathic porphyria; and in lead poisoning where rather large amounts of porphyrin are excreted, quite similar motor neuritis is encountered. As it is well known, for instance from the investigations reported by Rimmington, that on overdosage of sulfonamides often is accompanied by excretion of very large amounts of porphyrin, depending on the toxicity of the sulfonamide in question, it would be reasonable here too to consider whether the neurotoxic effect of the drug might not be brought about through a secondary porphyria.

ABSTRACTS — ANALYSES — REFERATE.

Francis Harbitz: »Sen-blødninger« i Hjernen med Hinner og deres Betydning i Ulykkestrygden og i Rettsmedicinen.

Communications published by the Norwegian Academy of Science; Dep. of Mathematics and Natural Science. No. 1. Oslo 1943. pp. 132.

For many years Professor Harbitz has taken an interest in the intracranial hemorrhages — especially on account of their role in forensic medicine and insurance. He has summed up his experiences in this field in the present book, which shows that he has preserved his great working capacity unimpaired at an advanced age. In this work he deals in particular with the »late hemorrhages« — a concept that was introduced by Bullinger in 1891 — which still are of considerable pathological and medicolegal interest, among other reasons because the pathogenesis of these hemorrhages often is obscure and their occurrence gives not infrequently rise to intricate lawsuits and insurance claims.

For introduction, Harbitz gives a review of the literature on late hemorrhages, and he rightly points out how Bullinger originally set up his concept merely on a slight foundation, on which account this concept always has been disputed ardently. On reading this chapter, indeed, one feels the aftermath of the well-known heated discussion between Harbitz and Berner concerning the traumatic intracranial hemorrhages. The outcome of this review of the literature is that late intracranial hemorrhages have to be taken as rare phenomena, and Harbitz emphasizes rightly that it is impossible to establish a certain limited interval within which these late hemorrhages must have occurred if they are to be recognized as being of traumatic origin. Nor is it always practicable to maintain the requirement concerning a positive history of a severe initial injury to the head and »bridge symptoms«.

The great difficulty lies in the differentiation of this lesion from spontaneous cerebral hemorrhage as well as from embolism, tumors, etc., and Harbitz emphasizes that the greatest caution has to be exercised in judging of each individual case.

Harbitz' own material comprises 58 cases which are divided into four groups: 1) a group of genuine late hemorrhages in the stricter sense of the term, *i. e.*, in the brain; 2) another group comprising late hemorrhages in the meninges; 3) traumatic hemorrhages due to lesions of the blood vessels (arteriosclerosis and syphilitic arteriitis); and 4) a group comprising cases of various diseases of the brain in which there was a positive history of traumatic injury to the head, and in which the disease was assumed to be of traumatic origin — in view of the late hemorrhage. In this classification Harbitz thus goes beyond the scope of his subject proper, but it has to be conceded that he is justified in doing so because it is difficult clinically and, to some extent, pathologic-anatomically too, to distinguish between the various forms of hemorrhage.

The first group, in which the hemorrhages are located in the brain, comprises 26 cases, 11 of which were examined post mortem. After a very thorough analysis of these cases, Harbitz arrives at the conclusion that only 1 case offered sufficient evidence to make it probable that the lesion really was a traumatic late hemorrhage. In 8 of the cases, however, the patients were found entitled to compensation because there was a positive history of a severe injury to the head and no other probable explanation could be

given of the origin of the hemorrhage. In the opinion of Harbitz, then, late traumatic hemorrhage into the brain is very rare, but it is necessary for practical reasons to maintain the concept. Through his case records Harbitz further illustrates that the question about late hemorrhage is raised not infrequently in cases of entirely different brain lesions.

The group of hemorrhages in the meninges comprises but a few cases, and here the author naturally deals especially with the subdural hematomas. Here one notices that in the course of time the author gradually has adopted the now prevailing view that the subdural late hematomas preponderantly are of traumatic origin. As to the subarachnoid hemorrhages, Harbitz thinks that most often they are spontaneous. But the decision of this question is very difficult because of the frequent presence of predisposing factors, especially aneurysms, in which cases even a small traumatic injury may elicit a hemorrhage which possibly may be followed by a recurrence (late hemorrhage).

Traumatic injuries may also have a marked influence on the course of preexisting diseases of blood vessels in the brain and may induce hemorrhage, occasionally with recurrence.

The whole work is stamped by the author's clear, stringent, trend of thought and sober criticism, and it has to be characterized as a very weighty contribution in the discussion of the present question.

W. Munch.

F. Brandt & K. Linderstrom-Lang: Videnskaben i Dag (Science of today), pp. 510, Schultz, Copenhagen, 1944, price 37.50 Danish crowns.

When, in contrast to many other popular-scientific publications, this work is to be mentioned in this journal, it is particularly for two reasons.

For one thing, all the 28 contributors are men of considerable scientific standing, and several of them are of international reknown, e.g., August Krogh, Øjvind Winge, Bengt Stromgren. Furthermore, this work illustrates how biology today occupies a central position in the total realm of science — between physics and chemistry on one side and sociology and humanistics on the other.

The first and larger section of the work, *Atom and Molecules*, brings some interesting chapters on subjects as the structure of the atom, quantum-theory the structure and transformation of the molecules, etc.

From this there is a gradual transition to the second section of the work, dealing with the cells and organisms. In this part we read about the structure of cells, embryology, investigations into heredity, the theory of evolution, palaeontology, proteins and enzymes, vitamins, hormones, and chemotherapeutics. After an intersection dealing with the developmental history of the sun and stars, the work concludes with a section on society and culture, treating, among other things, of ethnology, archeology, philology, religion, literature, sociology and psychology.

Within the rather narrow frame of this work, of course, it has been possible to throw only brief and apparently rather accidental light on the enormous subject under treatment. Naturally, it has not been practical to give a clear and at the same time comprehensive picture of the entire scientific, natural and humanistic, range of subjects from the atoms and molecules through the constitution of the living organisms to the first national and social formations in history, and, further, to the highly developed forms of culture within spiritual life. Still, the reading of this work gives a survey of recent research in its purely scientific form as conceived by men who have taken an active part in its development.

T. K.

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VOL. XXI

FASC. 4. 1944.

EINAR MUNKSGAARD · KØBENHAVN

MCMXLIV

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ACTA PATHOLOGICA ET MICROBIOLOGICA SCANDINAVICA

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In ACTA PATHOLOGICA ET MICROBIOLOGICA SCANDINAVICA only articles written by Scandinavian authors are published; they are issued in English, French or German, according to the author's desire.

Subscribers are requested to apply to *Ejnar Munksgaard*, Publisher, Copenhagen, Nørregade 6. One volume (generally 4 numbers, ca. 6—700 pages) is published every year with numerous supplements. Each volume costs 35 Danish crowns.

Dans ACTA PATHOLOGICA ET MICROBIOLOGICA SCANDINAVICA ne sont publiés que des articles écrits par auteurs scandinaves; selon leur désir, ils seront publiés en français, anglais ou allemand.

Pour les abonnements on est prié de s'adresser au éditeur, *M. Ejnar Munksgaard*, Copenhague, Nørregade 6. Prix par volume Cr. Dan. 35.—.

In ACTA PATHOLOGICA ET MICROBIOLOGICA SCANDINAVICA werden nur Artikel von skandinavischen Verfassern veröffentlicht; den Wünschen der Verfasser gemäss erscheinen sie in deutscher, englischer oder französischer Sprache. Zu beziehen von der Verlagsbuchhandlung *Ejnar Munksgaard*, Kopenhagen, Nørregade 6. Preis pro Band 35 dänische Kronen.

IN ACTA PATHOLOGICA ET MICROBIOLOGICA SCANDINAVICA optages Afhandlinger skrevet af 2 Ark (32 Sider). Manuskripter oversatte til Engelsk, Fransk eller Tysk, til en af

SEROLOGISCHE UND GENETISCHE UNTERSUCHUNGEN ÜBER SELTENE A-TYPEN BEI MENSCHEN

1. BLUTTYPUS A_3 .

Von Arne Gammelgaard.

(Eingegangen bei der Redaktion am 29. Mai 1943).

An die bekannten Versuche v. *Dungern's und Hirszfeld's* (5) über Absorption von Anti-A-Seren mit den Blk. verschiedener A-Individuen schloss sich jene Reihe von Untersuchungen, die zur Teilung der A-Gruppe in A_1 und A_2 führten, indem nachgewiesen wurde, dass innerhalb der A-Gruppe 2 allem Anschein nach quantitativ verschiedene A-Rezeptoren vorkommen, die sich auf Basis je ihres Gens allelomorph mit den übrigen Bluttypengenen vererben.

1936 veröffentlichte *Friedenreich* (8, 9) eine Reihe Untersuchungen über einen sehr schwachen A-Rezeptor, den er A_3 nannte. Die Untersuchung enthielt die Typenbestimmung von 6 Familien, in denen 46 A_3 und 3 A_3B nachgewiesen wurden. Durch die Familienuntersuchung wurde es wahrscheinlich gemacht, dass sich der A_3 -Typus auf Basis eines 3. allelomorphen A-Gens vererbe. Serologisch ist der A_3 -Rezeptor durch eine viel schwächere Agglutination als A_2 charakterisiert, schwächer als A_2B oder höchstens ebenso stark. Aber nebst der schwachen Agglutination waren die A_3 -Blk. auch durch ein eigentümliches Agglutinationsbild auf dem Objektträger charakterisiert, indem langsam nicht besonders kleine, aber brüchige Agglutinate zwischen vollständig unagglutinierten Blk. auftraten. Die Absorptionsfähigkeit lag bei den A_3 -Blk. zwischen der der A_2 und A_2B , d. h. sie absorbierten schwächer als die A_2 -Blk., aber stärker als die A_2B -Blk. Die A_3 -Mitglieder einer einzelnen Familie (Familie 1 in *Friedenreich's* Artikel; im folgenden Familie J. benannt) wiesen die Eigentümlichkeit auf, dass die Blk., obwohl sie auf dem Objektträger eine kräftigere Agglutination zeigten als die übrigen A_3 , doch weniger absorbierten als diese, indem ihre Absorptionsfähigkeit schwächer war als bei A_2B . *Friedenreich* erwähnt in einem späteren Artikel, dass diesem A_3 möglicherweise eine besondere Variante des A_3 -Gens zugrundeliege. In einer anderen Familie (Familie 3 in *Friedenreich's* Artikel, im folgenden Familie H. benannt) kam eine Abweichung von der Alleltheorie vor: ein O-Sohn eines Vaters, dessen Genotyp nach dem System A_2A_3 sein sollte. Die Abweichung wäre durch Illegitimität erklärlich. *Friedenreich* stellte auch die Hypothese auf, dass A_3 in dieser Familie auf dem Einfluss eines rezeptor-modifizierenden, abschwächenden Gens auf das A_2 -Gen beruhe, wonach der Vater genotypisch ein A_2O + einem modifizierenden Gen darstelle und somit instande sei, A_3 - und O-Kinder zu bekommen. *Friedenreich* betonte

diese Möglichkeit, weil die Blk. aller A_2 -Personen dieser Familie nicht nur mit α_2 reagierten (Anti-O haltiges Ochsen Serum), sondern auch mit α_1 eine schwache Reaktion auswiesen und demnach von den »normalen« A_2 -Blk. abwichen. Diese A_2 -Form sollte genotypisch ein A_1 O + dem modifizierenden Gen darstellen.

1910 legten *Gammelgaard und Marcussen* (14) eine Untersuchung über einen noch schwächeren A-Rezeptor vor, den sie A_4 benannten, da er eine deutliche schwächere Rezeptorfähigkeit aufwies als A_3 und im übrigen keine der für A_3 charakteristischen Merkmale trug. Die Untersuchung enthielt die Typenbestimmung einer Familie von 64 Mitgliedern, von denen 24 den schwachen A-Rezeptor hatten. Nach der Familienuntersuchung scheint die Annahme gerechtfertigt, dass sich A_4 auf Basis eines selbständigen allelomorphen A-Gens entwickelte.

Fischer und Hahn (6) haben 1935 ein schwaches A an einem mit langwierigem Fieber behafteten Patienten beschrieben. Der A-Rezeptor war deutlich schwächer als der A-Rezeptor bei A_2 B. *Friedenreich* fuhr in seinen Untersuchungen an, dass *Fischer und Hahn's* »schwaches A« sicher A_3 war, obwohl kein Agglutinationsbild vorlag wie das von *Friedenreich* für A_3 als charakteristisch bezeichnete.

Hirszfeld und Amzel (21) haben 1940 ein schwaches A beschrieben, das sie A_4 benannten. Der schwache A-Rezeptor wurde an einem Kind nachgewiesen, ferner an dessen durch Paternitätsklage anerkanntem Vater sowie an einem Bruder des letzteren. Die Bezeichnung A_4 ist unabhängig von *Gammelgaard und Marcussen's* Bericht. Der A_4 -Rezeptor wird als 8-mal schwächer als A_2 bezeichnet. Die von *Friedenreich* für A_3 beschriebene charakteristische Agglutination wird nicht erwähnt, aber *Hirszfeld* stellt teilweise hypothetisch 3 Typen A_3 , A_4 und A_5 auf, die sich durch ihre Rezeptorstärke unterscheiden, A_4 und A_5 sind jedoch auch dadurch charakterisiert, dass bei A_4 kein Anti A (irreguläres α_1) im Serum vorkommen darf, was dagegen bei A_5 der Fall ist: eine Charakteristik, die, wie *Dahr* (4) anführt, unhaltbar ist, da irreguläres α_1 (Anti A) ja schon bei A_2 und A_2 B vorkommt.

O. Hartmann u. a. m. (19) fanden 1940 unter 2–3000 Typenbestimmungen einen schwachen A-Rezeptor, der mit A_3 bezeichnet wurde. *Hartmann* fuhr an, dass von einer charakteristischen Agglutination wie von *Friedenreich* angegeben keine Rede gewesen sei, und meint, dass auch letztere als eine schwache »normale« Agglutination aufzufassen sei.

Morawiecki (27) beschreibt 1941 ein schwaches A_3 , das wie bei *Hirszfeld* und *Amzel* 8-mal schwächer ist als A_2 und deshalb auch A_4 benannt wird.

Kammann (23) hat 1942 ein schwaches AB beschrieben, das er als A_3 B auffasst. Es wird mit *Fischer und Hahn's* »schwachem A« verglichen und bedeutend schwächer befunden. Von einer charakteristischen Agglutination wie bei *Friedenreich* wird nichts angeführt.

Wiener und Silvermann (34) haben 1941 an einer Negerin und ihrem 1-jährigen Kind den A_3 -Typ mit allen den von *Friedenreich* für A_3 beschriebenen charakteristischen Zügen nachgewiesen, speziell mit dem Agglutinationsbild auf dem Objektträger.

Endlich hat *Dahr* (4) 1942 ein durch das eigentümliche Agglutinationsbild charakterisiertes A_3 gefunden.

Es liegen somit von verschiedenen Seiten Berichte vor, die deutlich zeigen, dass die Teilung der A-Gruppe mit der Aufstellung der A_1 - und A_2 -Typen bei weitem noch nicht abgeschlossen ist.

Um neue A-Typen in die Reihe einschalten zu können, scheint es mir nach den bei den Untersuchungen über A_1 und A_2 gemachten Erfahrungen, dass folgende Bedingungen erfüllt werden müssen:

1) Der neue A-Typ muss sich quantitativ (eventuell auch qualitativ) klar von den übrigen Typen unterscheiden lassen.

2) Er muss sich auf Basis eines selbständigen allelomorphen Gens vererben.

Ich werde im folgenden über Untersuchungen berichten, die es nach dem oben Angeführten wahrscheinlich machen, dass noch 3 A-Typen A_3 , A_4 und A_5 existieren.

Das Material ist dadurch zuwege gebracht worden, dass von ca. 80.000 Typenbestimmungen, die im Universitätsinstitut für gerichtliche Medizin und im Staatlichen Seruminstitut vorgenommen wurden, alle Fälle schwacher A-Rezeptoren herausgenommen wurden und zur Beleuchtung der genetischen sowie der serologischen Verhältnisse eine Typenbestimmung an allen lebenden Familienmitgliedern jener Personen angestellt wurde, bei denen ein schwacher A-Rezeptor festgestellt wurde.

Der Bluttypus A_3 .

Das Material umfasst 11 A_3 -Fälle aus 20.000—25.000 im Universitätsinstitut für gerichtliche Medizin in den Jahren 1936—40 vorgenommenen Typenbestimmungen. Dazu kommen 23 A_3 (16 A_3 und 7 A_3B) aus 52.000 Typenbestimmungen am Staatlichen Seruminstitut in den Jahren 1938—40. Die A_3 -Häufigkeit ist in beiden Fällen einigermaßen gleich, und A_3 macht demnach ca. 1 ‰ der A-Gruppe aus.

Es wurden an 26 A_3 -Familien Typenbestimmungen vorgenommen und hierbei insgesamt 203 A_3 (170 A_3 und 33 A_3B) festgestellt.

Die Typenbestimmung der Familienmitglieder ist auf verschiedenen Reisen ringsum im Land vorgenommen worden. Die Blutproben sind in den allermeisten Fällen durch Stich in den lobulus auriculæ entnommen und in physiologischer Kochsalzlösung mit einem Zusatz einer 3,5 %-igen Natriumcitratlösung gesammelt worden. Die Proben wurden noch am selben Tag, in einzelnen Fällen am Tag nach der Entnahme untersucht. Die Bestimmung von A_3 geschah in einer Auswahl von Iso-B-Seren und Anti-A-Immun-Seren. Zum Vergleich wurde Blut von ♀ H. Typus A_2 , ♂ Kr. Typus A_2B und von ♂ W. Typus A_3 oder ♂ F. Typus A_3 in koaguliertem Zustand mitgebracht. Die Blk. dieser »Standardblutproben« konnten erfahrungsgemäss bei Aufbewahrung der koagulierten Blutproben in abgekühlten Thermosflaschen die 8 Tage, welche die Reise normal dauerte, ihre Agglutinabilität unverändert bewahren. Es wurden mit den Blk. mehrerer A_2 - und A_3 -Individuen innerhalb derselben Familie Absorptionsversuche angestellt und mit solchen der Standardproben verglichen. Die A_1 — A_2 Bestimmung ist mit einem mit A_2 -Blk. absorbierten Iso-B-serum und einem mit A_1B -Blk. absorbierten Anti-O-hältigen Ochsen Serum vorgenommen worden, und zwar nach der von Friedenreich und Zacho gegebenen Anweisung. Alle Blk. sind ferner nach dem M.N. System bestimmt worden, teils im Hinblick auf eine weitere Sicherstellung der Paternität in zweifelhaften Fällen, teils zur Kontrolle, dass die Blk. nicht aus irgend einem Grund eine allgemeine Schwächung erfahren hatten.

Durch die Untersuchung der oben genannten 170 A_3 und 33 A_3B konnten die in der Folge beschriebenen serologischen Verhältnisse festgestellt werden.

Der A_3 -Rezeptor ist (bei einer Mischung von 1 Tr. 3 % Blk.suspension und 1 Tr. Serum) vor allem durch eine schwache und eigenartige Agglutination auf dem Objektträger charakterisiert, indem sich im Laufe kürzerer oder längerer Zeit, je nach der Stärke des angewandten Serums, kleine scheinbar gleichartige Agglutinate bilden, die sich allmählich zu grösseren vereinigen und sich zentral lagern, so dass das charakteristische Bild entsteht, indem die zentral gelagerten grösseren Agglutinate von einigen kleineren umgeben sind und sich diffus »Flocken« von unagglutinierten Blk. gebildet haben. Diese Mischung von grösseren Agglutinaten und unagglutinierten Blk. hält sich selbst bei längerer Beobachtung und ist nicht von der Stärke des angewandten B-Serums abhängig indem nämlich die Mischung bestehen bleibt, wenn auch je nach Anwendung von Seren verschiedener Stärke das Verhältnis zwischen agglutinierten und nicht-agglutinierten Blk. sich verschiebt.

Die Blk. verschiedener A_3 -Individuen sind in ihrem Verhalten zu ca. 30 auserwählten Iso-B-Seren (Titer 64—1000) untersucht worden, und eine Reihe Iso-B-Seren wurde den täglichen Proben entnommen. In allen Seren zeigten die A_3 -Blk. das charakteristische Agglutinationsbild, selbst im Serum mit dem Titer 1000 sah man deutlich die Mischung agglutinierten und unagglutinierten Blk.

Bei Agglutinationsuntersuchungen nach dem Zentrifugierungsverfahren a. m. Schiff oder bei 12—18-stündigem Stehenlassen im Reagensgläschen sieht man ebenfalls die charakteristische Mischung agglutinierten und unagglutinierten Blk.

Die Agglutination der A_3 -Blk. muss entsprechend dem eigenartigen Agglutinationsbild, wo sich einige Blk. unagglutiniert halten, als schwache Agglutination bezeichnet werden. Vergleicht man die Agglutination von A_3 -Blk. mit der von A_2 - und A_2 B-Blk. in verschiedenen Iso-B-Seren und Anti-A-Immunseren, finden wir bei Anwendung des Objektträgerverfahrens mit Ablesung nach 15 Minuten den in Tabelle 1 aufgezeigten Tatbestand.*)

Tabelle 1.
Agglutination von A_2 -, A_2 B- und A_3 -Blk. in 6 Iso-B-Seren und 1 Anti-A-Immunserum.

Blutk.	Iso-B-Seren						Immunserum
	J. (1024)	U. (512)	V. (256)	Johs. (128)	E. A. (32)	M. (16)	K. 19 (512)
A_3 (H.)	++++	++++	++++	+++	+++	++	++++
A_2 B (Kr.) ..	++++	++++	+++	++	+	Sp	++++
A_3 (W.)	[+(+)]	[+(+)]	[+]	[+]	[±]	[±]	[±]

*) Agglutinationsangaben gelten in der Folge, wenn nichts anderes angegeben, für Objektträgerreaktion mit Ablesung nach 15 Minuten.

Der Titer der angewandten Sera mit Bezug auf A_1 -Blk. variiert von 1000—16 und ist in Klammer unter den Serumbezeichnungen angeführt. Zur Bezeichnung des charakteristischen Agglutinationsbildes der A_3 -Blk. ist die eckige Klammer $[\]$ angewendet.

Es geht daraus hervor, dass die A_3 -Blk. in den starken Seren bedeutend schwächer reagieren als die A_2B -Blk., aber während die A_2B -Blk. einen deutlichen Fall der Agglutinationsintensität bis zur Reaktion Sp. in den schwachen Seren aufweisen, variiert die Agglutinationsstärke der A_3 -Blk. nur wenig mit der zunehmenden Schwäche der Seren, und es zeigt sich das Eigentümliche, dass die A_3 -Blk. im schwachen Serum M deutlich kräftiger agglutinieren als die A_2B -Blk. Betrachtet man das Verhältnis zwischen der Agglutinationsstärke von A_2B und A_3 im Immunserum K. 19 und in den Iso-Seren, ist der Unterschied auffällig. In K. 19 agglutiniert A_2B maximal wie in den B-Seren U. und V., aber die A_3 -Agglutination ist in K. 19 nur $[\pm]$, während sie in U. und V. $[+(+)]$ bzw. $[+]$ ist.

Dieser auffällige Unterschied der Avidität zwischen A_3 -Blk. und Immunantistoffen einerseits und A_3 -Blk. und Iso-Antistoffen andererseits gilt nicht nur für ein einzelnes Immunserum. Ca. 30 Immunsere, die durch Immunisierung von Kaninchen mit A_1 - oder A_2 -Blk. hergestellt wurden, zeigten dasselbe Bild, und ebenso haben 12 Immunsere, die durch Immunisierung mit A_3 -Blk. hergestellt wurden, dieselbe schwache Agglutination mit A_3 -Blk. ergeben.

Diese auffällig schwache Agglutination in Immunsere ist ein wichtiges Merkmal des A_3 -Typs, und wichtig auch durch Abweichungen zwischen A_3 und andern schwachen Rezeptoren wie A_4 und A_5 , wie später gezeigt werden wird.

Die verhältnismässig geringen Schwankungen der Agglutinationsstärke der A_3 -Blk. in Iso-Seren mit verschiedenem Titer findet man auch bei quantitativen Messungen der Rezeptorstärke, durch Bestimmung der schwächsten Serumkonzentration, in der A_3 -Blk. gerade noch agglutiniert werden.

Tabelle 2.

Iso-B-Serum V. austitriert gegenüber A_2 -, A_2B - und A_3 -Blk.

Blutk.	B-Serum V.								
	$1/1$	$1/2$	$1/4$	$1/8$	$1/16$	$1/32$	$1/64$	$1/128$	$1/256$
A_2 (H.).....	++++	++++	+++	+++	++	+(+)	+	Sp	0
A_2B (Kr.) ..	++++	+++	++	+	\pm	0			
A_3 (W.)	$[(+)]$	$[+]$	$[\pm]$	$[\pm]$	$[\pm]$	Sp	0	0	0

Es erhellt aus Tabelle 2, dass die A_3 -Agglutination eine verhältnismässig lange Reihe Agglutinationen bildet, die bei steigender Serum-

verdünnung nur schwach abnehmen, während A_2B einen im Verhältnis hierzu kräftigen Fall aufweist und ausserdem anscheinend schwächer ist als A_3 , da die Agglutination in der Verdünnung 1/16 aufhört, während die A_3 -Blk. noch in der Verdünnung 1/32 eine schwache, aber unverkennbare Agglutination aufweisen, und dies stimmt mit dem Resultat der Untersuchungen über mehrere Seren verschiedener Stärke, wie in Tabelle 1 gezeigt, gut überein.

Wie von Friedenreich erwähnt, bestätigen Absorptionsversuche, dass der A_3 -Rezeptor kräftiger als der A_2B -, aber schwächer als der A_2 -Rezeptor ist. Meine Absorptionsversuche, die mit Blk. von 75 verschiedenen A_3 -Individuen vorgenommen worden sind, stimmen mit den Untersuchungen Friedenreichs überein. Die Absorptionsversuche sind mit 1/2, 1/5 und 1/15 Volumen A_3 -Blk. ausgeführt, verglichen mit Standard A_2 - und A_2B -Blk. Tabelle 3 zeigt einen solchen Absorptionsversuch.

Tabelle 3.

Iso-B-Serum O. K. absorbiert mit $1/2$, $1/5$ und $1/15$ Vol. A_2 -, A_2B - und A_3 -Blk. Serum austitriert gegenüber A_1 -Blk.

	Serumverdünnungen							
	$1/1$	$1/2$	$1/4$	$1/8$	$1/16$	$1/32$	$1/64$	$1/128$
B-Serum O. K. unabsorb...	++++	++++	+++	++	(+)+	+	±	0
„ abs. m. $1/2$ Vol. A_2 (H.)	++	+	±	0				
„ „ „ „ „ A_2B (Kr.)	+++	+++	++	+	±	0		
„ „ „ „ „ A_3 (W.)	++	++	+	Sp	0			
„ „ „ „ „ $1/5$ „ A_2	+++	++	+	Sp	0			
„ „ „ „ „ A_2B	++++	+++	++	+	±	Sp	0	
„ „ „ „ „ A_3	+++	+++	++	±	0			
„ „ „ „ „ $1/15$ „ A_2	+++	++	(+)+	+	0			
„ „ „ „ „ A_2B	++++	+++	+++	++	+	±	0	
„ „ „ „ „ A_3	+++	+++	++	+	±	0		

Nebst der Feststellung der Absorptionsfähigkeit des A_3 -Rezeptors durch die Absorptionsversuche war es bei der Untersuchung der absorbierten Sera auch von Interesse zu erfahren, ob bei Absorption mit A_2B - oder A_3 -Blk. eine elektive Titerreduktion nachgewiesen werden konnte, ähnlich der von Landsteiner und Witt (26) nachgewiesenen Titerreduktion für A_2 bei Absorption von B- und O-Seren mit A_2 -Blk. oder mit kleinen Mengen A_1 -Blk., wie später von Friedenreich und Worsaae (12) gezeigt. D. h. existiert im Anti-A-Agglutinin eine Fraktion, die elektiv gegen A_1 - und A_2 -Blk. gerichtet ist? Zur Klärung dieser Frage sind die absorbierten Sera nicht nur den A_1 - und A_2 -Blk., sondern auch den A_3 -Blk. gegenüber austitriert worden, und es wurden

Absorptionsversuche schwacher Sera mit kleinen Volumina A_2B - und A_3 -Blk. mit nachfolgender Austitrierung der absorbierten Sera den A_2 -, A_2B - und A_3 -Blk. gegenüber vorgenommen, und zum Vergleich wurden teils Verdünnungen der zur Absorption verwendeten Sera, teils noch schwächere Sera angewendet.

Bei diesen Versuchen hat sich gezeigt, dass durch Absorption mit A_2B - oder A_3 -Blk. keine elektive Titerreduktion für A_3 erzielt werden kann. Durch passende Absorption kann ein Serum hergestellt werden, wo A_2B nicht agglutiniert wird, A_3 schwach und A_2 kräftig, dem Agglutinationsverhältnis in einem schwachen Serum oder in der Verdünnung eines kräftigen Serums entsprechend. Tabelle 4 gibt ein Beispiel dafür.

Tabelle 4.

Iso-B-Serum E. A. absorbiert mit $1/10$, $1/30$ und $1/90$ Vol. A_2 -, A_2B - und A_3 -Blk. Die absorbierten Sera mit A_2 -, A_2B - und A_3 -Blk. geprüft.

Blutk.	Iso-B-Serum E. A.									
	un-abs.	abs. m. $1/10$ Vol.			abs. m. $1/30$ Vol.			abs. m. $1/90$ Vol.		
		A_2	A_3	A_2B	A_2	A_3	A_2B	A_2	A_3	A_2B
A_2 (H.)	+++	Sp	+	++	\pm	++	+++	+(+)	+++	+++
A_3 (L.)	[\pm]	0	0	Sp	0	0	[\pm]	0	[\pm]	[\pm]
A_2B (Kr.) ..	+	0	0	0	0	0	Sp	0	Sp	\pm

In Immunsereen ist die Absorptionsfähigkeit des A_3 -Rezeptors gleich A_2B oder etwas schwächer, der schwachen Agglutination entsprechend, die sich bei quantitativer Bestimmung der Rezeptorstärke durch Austitrierung in Verdünnungen bis 1 Stufe unter A_2B ergibt. (Siehe Tabelle 5).

Tabelle 5.

Blutk.	Immuns erum K. 19							
	1/1	1/2	1/4	1/8	1/16	1/32	1/64	1/128
A ₂ (H.)	++++	++++	++++	++++	+++	++	+	0
A ₂ B (Kr.)	++++	++++	++++	+++	++	+	0	
A ₃ (W.)	[±]	[±]	[±]	Sp	Sp	0		

Bei der Untersuchung der 33 A_3B -Fälle, die das Material umfasst, zeigt es sich, dass B eine hemmende Wirkung auf den A_3 -Rezeptor ungefähr im selben Verhältnis ausübt, wie A_2 von B gehemmt wird. Die hemmende Wirkung auf A_3 war in allen Fällen konstant.

Tabelle 6.

Iso-B-Serum V. austitriert gegenüber A_2 -, A_2B -, A_3 - und A_3B -Blk.

Blutk.	$1/1$	$1/2$	$1/4$	$1/8$	$1/16$	$1/32$	$1/64$	$1/128$
A_2 (H.).....	++++	++++	+++	+++	++	+(+)	+	Sp
A_2B (Kr.)	++++	+++	++	+	\pm	0		
A_3 (W.)	[+(+)]	[+]	[+]	[\pm]	[\pm]	Sp	0	
A_3B (L.).....	[+]	[\pm]	[\pm]	0				

Friedenreich und Waaler (11) machen darauf aufmerksam, dass Landsteiner und Levine (25), als sie 500 Serumproben auf irreguläres α_1 untersuchten, dieses bei A_2B ebenso häufig vorfanden wie bei A_2 , obwohl A_2 10-mal so häufig vorkommt wie A_2B . Die Autoren machen deshalb auf die Möglichkeit eines häufigeren Vorkommens von irregulärem α_1 bei schwachen A-Rezeptoren aufmerksam. Einen Fingerzeig in dieser Richtung sahen Friedenreich und Waaler auch darin, dass in einem Falle mit besonders kräftigem α_1 der A-Rezeptor deutlich schwächer war als Standard A_2 -Blk., und in einem Falle sogar schwächer als A_2B .

Friedenreich fand bei Zimmertemperatur in keinem Falle bei A_3 oder A_3B ein irreguläres α_1 . Nur in ganz einzelnen Fällen konnte ein bei 2—5° wirksames irreguläres α_1 nachgewiesen werden, und unter den 3 A_3B fand er ein bei 10° wirksames α_1 .

Ich habe das Serum von 50 Individuen des Typus A_3 und von 9 des Typus A_3B untersucht und in keinem Falle bei Zimmertemperatur ein irreguläres α_1 angetroffen.

Der A_3 -Rezeptor scheint somit nicht die Bildung eines irregulären α_1 zu fördern, im Gegensatz zu A_2B , wo irreguläres α_1 mit einigen Prozenten nachzuweisen ist (eigene Erfahrungen). Pietrusky (29) erwähnt 25 %, aber es muss sich dann sicher um niedrige Temperaturen handeln.

Nach Untersuchungen von Kemp (24), Thomsen (32) und Worsaae (35) entwickelt sich der A-Rezeptor allmählich während des Fötallebens und nach der Geburt, und zwar so, dass der A_2 -Rezeptor meistens voll entwickelt ist, wenn das Kind 4—6 Monate alt ist, der A_2 -Rezeptor gewöhnlich etwas später. Ich habe 3 Kinder (von 3, 5 bzw. 12 Monaten) des Typus A_3 untersucht und gefunden, dass bei allen diesen Kindern der A_3 -Rezeptor durch das eigenartige Agglutinationsbild und die schwache Reaktion in Immunsereen nachgewiesen werden konnte. Am schwächsten ist der Rezeptor bei dem 2-monatigen, kräftiger bei dem 5-monatigen, aber zur selben Höhe wie bei Erwachsenen erst bei dem 12-monatigen Kind entwickelt.

Die Blk. aller A_3 -Individuen sind in Anti-O-hältigem Ochsen-serum untersucht worden, verglichen mit Standard A_2 - und O-Blk.

Ich finde hier in Übereinstimmung mit den Resultaten Friedenreichs, dass die A_3 -Blk. in Anti-O-hältigem Ochsen Serum kräftiger agglutiniert werden als A_2 -Blk., aber schwächer als O-Blk. Nach den verschiedenen theoretischen Erwägungen über die O-Substanz entspricht dieser Befund den Erwartungen (*Dahr* (3), *Friedenreich* (12), *Greenfield* (15), *Hirszfeld* (20, 22), *Schiff* (30), *Thomsen* (33)).

Indessen kommen im Familienmaterial 6 Individuen des Typus A_2 vor, deren Genotypus sicher mit A_2A_3 bestimmt werden kann. 4 davon sind Eltern, die A_3 -Familien angehören und mit Individuen des Typus O oder A_1 verheiratet sind; ihre Kinder haben ausschliesslich den Typus A_2 und A_3 (und in dem einen Falle A_1). Die übrigen 2 sind Kinder der Elternkombination $A_3B \times A_2$.

Blk. dieser 6 A_2 -Individuen agglutinieren in Anti-O-hältigem Serum mit derselben Intensität wie Standard A_2 -Blk., was darauf schliessen lässt, dass die O-Substanz nicht ausschliesslich an das O-Gen des heterozygoten A_2O geknüpft ist (*Dahr*, *Friedenreich*, *Greenfield*, *Schiff*). Sowohl *Hirszfelds* Mutationstheorie sowie auch seine Verdrängungshypothese sind mit obigem verträglich, aber wenn wir zu den Typen A_4 und A_5 gelangen, decken sich die Theorien doch nicht ganz mit den Tatsachen, da die A_4 -Blk. in Anti-O-Serum mit derselben Stärke agglutinieren wie A_3 -Blk., während A_5 -Blk. nur wie A_2 -Blk. agglutinieren.

Erörterung über das Entstehen des charakteristischen Agglutinationsbildes der A_3 -Blk.

Die eigentümliche Erscheinung, dass bei der Agglutination von A_3 -Blk. selbst in den agglutininreichsten B-Seren doch immer nicht-agglutinierte Blk. auftreten und dass sich die Agglutination dadurch im Verhältnis zur Agglutination der A_2 - und A_2B -Blk. als schwach erweist, dass der A_3 -Rezeptor bei Austitrierung grössere Empfindlichkeit erweist als bei A_2B und bei Absorptionsversuchen auch eine grössere Bindungsfähigkeit, könnte durch eine Mischung von Blk. mit einer Rezeptorstärke ungefähr wie A_2 -Blk. und solchen mit schwächerem oder vielleicht nicht nachweisbarem A-Rezeptorgehalt erklärt werden. Durch Herstellung verschiedenartiger Mischungen von A_2 - und O-Blk. ist es auch möglich, damit ein Agglutinationsbild desselben Aussehens wie das der A_3 -Blk. auf die Platte zu bekommen. Diese Imitation hat aber nur eine begrenzte Ähnlichkeit, indem eine Agglutinationsuntersuchung in starken B-Seren mit Anwendung der Reagensglasmethode zeigt, dass A_2 -Blk. in einem grossen Klumpen agglutinieren, der als solcher unter den nicht agglutinierten O-Blk. herausgeschüttelt wird. Es muss sich deshalb bei den A_3 -Blk. eher um einen allmählichen Übergang von Blk., die mit einem Rezeptor von der Stärke der A_2 -Blk. ausgestattet sind, und solchen mit schwächerem, ja vielleicht ganz fehlendem A-Rezeptorgehalt handeln: dann wird es bei der Agglutination selbst in den stärksten Seren Blk. geben, die

maximal agglutinieren, solche, die schwach, und endlich solche, die gar nicht agglutinieren. Diese Annahme wird durch die folgenden Versuche bekräftigt.

Die Versuche sind darauf angelegt, die Blk. zu untersuchen, die im Testserum unagglutiniert bleiben. Dabei ist das im folgenden als Schüttelversuch bezeichnete Verfahren angewendet worden.

Beim Schüttelversuch verfährt man wie bei einem gewöhnlichen Absorptionsversuch, indem man einem cm^3 B-Serum $\frac{1}{3}$ Vol. sorgfältig gewaschene und kräftig zentrifugierte A_3 -Blk. zusetzt. Nach $\frac{1}{2}$ -ständiger Bindung bei Zimmertemperatur wird das Glas vorsichtig zentrifugiert, z. B. durch 2000 Umdrehungen in einer Minute. Nach Herauspipettierung von Serum werden vorsichtig längs der Wand des Glases z. B. 2 cm^3 Kochsalzlösung zugesetzt. Man kann nunmehr durch vorsichtiges Schütteln des Glases einige unagglutinierte Blk. herausschütteln, die sich wie eine »Wolke« in der Kochsalzlösung erheben. Die dadurch entstandene Blk.-suspension der herausgeschüttelten Blk. wird zweimal in Salzwasser gewaschen. Nur eine sehr dünne Suspension kann auf diese Weise zu Stande kommen, da das Ausschütteln sehr vorsichtig gemacht werden muss, um zu vermeiden, dass gleichzeitig feine Agglutinate heraussgeschüttelt werden.

Wenn dann die herausgeschüttelten Blk. in dem Serum, das zum Schüttelversuch angewendet wurde, untersucht und mit einer Suspension »unbehandelter« A_3 -Blk. verglichen werden, dann zeigt es sich, dass die ausgeschüttelten Blk. inagglutinabel sind, wie aus Tabelle 7 hervorgeht, die Schüttelversuche im Serum E. A. (Titer 32) und Serum U (Titer 512) betrifft.

Tabelle 7.

Blutk.	B-Serum E. A. (Titer 32)	Blutk.	B-Serum U. (Titer 512)
Ausgeschüttelte A_3 -Blk. aus Serum E. A...	0	Ausgeschüttelte A_3 -Blk. aus Serum U...	0
»Unbehandelte« A_3 -Blk.	[\pm]	»Unbehandelte« A_3 -Blk.	[+(+)]

Es fragt sich nunmehr, ob die ausgeschüttelten Blk. entweder Blk. ohne A-Rezeptor oder solche mit einer besonderen Art A-Rezeptor sind oder etwa Blk., die aus irgend einem Grund inagglutinabel sind. Morphologisch bieten die ausgeschüttelten Blk. keine Eigentümlichkeiten dar.

Bei Untersuchungen in einer Reihe verschiedener Iso-B-Seren, die teils schwächer, teils stärker sind als das beim Schüttelversuch angewendete, agglutinieren die ausgeschüttelten Blk. in den stärkeren Seren und nicht in den schwächeren, und die Agglutination in den stär-

keren Seren ist in Bezug auf die Stärke vom Titer des Serums abhängig. Ferner agglutinieren die ausgeschüttelten Blk. in dem charakteristischen Bild im selben Sinne wie die unbehandelten Blk., aber schwächer als diese, was aus der Tabelle 8 hervorgeht.

Tabelle 8.

Blutk.	B-Sera.					
	J. (1024)	U. (512)	V. (256)	Johs. (128)	E. A. (32)	O. K. (16)
»Unbehandelte« A ₃ -Blk.	[+(+)]	[+(+)]	[+]	[+]	[±]	[±]
A ₃ -Blk. ausgesch. aus Serum E. A.	[+]	[+]	[±]	[±]	0	0
A ₃ -Blk. » » » U...	[±]	0	0	0	0	0

Die ausgeschüttelten Blk. zeigen demnach eine bedeutend schwächere Agglutinationsfähigkeit als die unbehandelten, und zwar eine um so schwächere, je kräftiger das zum Schüttelversuch angewendete Serum war. Dass die Agglutination der ausgeschüttelten Blk. nur von der Stärke (dem Titer) des zur Agglutination angewendeten Serums abhängig ist, kann gezeigt werden, wenn man den Schüttelversuch mit einem Serum vornimmt, das z. B. auf 1/4 verdünnt ist. Die von hier herausgeschüttelten Blk. werden im unverdünnten Serum agglutinieren, aber schwächer als »unbehandelte« Blk.

Hieraus erhellt, dass es durch Schüttelversuche mit A₃-Blk. möglich ist, Blk. auszuschcheiden, bei denen die Empfindlichkeit des Rezeptors vom Unnachweisbaren in B-Seren fast zur selben Stärke wie bei unbehandelten A₃-Blk. variiert.

Betrifft nun diese Eigenschaft speziell die A₃-Blk. und gilt sie für diese im allgemeinen, oder haftet sie nur am A-Rezeptor? Schüttelversuche mit A₁-, A₂- und B-Blk. sind nur in schwachen oder verdünnten Seren möglich, wo die Agglutination so schwach ist, dass sie mit: + oder: ± bezeichnet werden muss, d. h. dass sowohl agglutinierte als auch unagglutinierte Blk. vorkommen. Die bei einem solchen Versuch ausgeschüttelten Blk. agglutinieren auf dem Objektträger mit derselben Geschwindigkeit und Intensität wie »unbehandelte« Blk., und es kann auch nicht durch Austitrierung ein Unterschied der Rezeptorstärke nachgewiesen werden. Die Eigenschaft scheint somit speziell an A₃-Blk. geknüpft zu sein.

Wie früher erwähnt, ist die Agglutination der A₃-Blk. in Anti-O-Seren eine »normale Agglutination«. Alle untersuchten A₃-Fälle sind nach dem MN-System in Kaninchenimmunseren bestimmt, die durch Immunisierung mit OM- bzw. ON-Blk. hergestellt waren. Die Agglutination war in diesen Seren bei allen A₃-Blutproben von derselben Intensität und dem gleichen Aussehen wie die der Kontroll- M- und N-Blk., d. h. nicht schwächer und ohne das eigentümliche Aggluti-

nationsbild. Durch Schüttelversuche in Anti-O-Seren und in Anti-M- und N-Seren war es nicht möglich, einen Unterschied zwischen der Agglutinabilität der ausgeschüttelten und der unbehandelten Blk. festzustellen. Die verschiedenartige Agglutinationsfähigkeit der A_3 -Blk. scheint demnach allein an den A-Rezeptor geknüpft zu sein. Dies geht ausserdem noch daraus hervor, dass die aus einem starken B-Serum ausgeschüttelten Blk. mit derselben Geschwindigkeit und Intensität agglutinieren wie unbehandelte Blk. in Anti-O-, in Anti-M- und in Anti-N-Seren.

Man muss nunmehr annehmen, dass das eigentümliche Agglutinationsbild der A_3 -Blk. auf Unterschiede der A-Rezeptorstärke der Blk. zurückzuführen ist, was ferner noch durch Absorptionsversuche gezeigt werden kann. Durch Ausschütteln aus mehreren Gläsern mit 1 cm³ Serum und 0,4 cm³ Blk. gelang es, im ganzen 0,04 cm³ Blk. herauszubekommen. Zum Schüttelversuch wurde ein kräftiges B-Serum angewendet, um die am schwächsten mit Rezeptor ausgestatteten Blk. zu erhalten, aber dadurch können auch nur sehr wenige Blk. aus jedem Glas ausgeschüttelt werden. Den 0,04 cm³ ausgeschüttelten Blk. wird 0,2 cm³ B-Serum E. A. zugesetzt, und zum Vergleich wird 0,2 cm³ E. A. mit 0,04 cm³ unbehandelten A_3 -Blk. absorbiert. Die absorbierten Serumportionen sind sowohl gegenüber A_2 - als A_3 -Blk. autitriert. Das Resultat ist in Tabelle 9 angeführt.

Tabelle 9.

	Blk.	Serumverdünnungen				
		$\frac{1}{1}$	$\frac{1}{2}$	$\frac{1}{4}$	$\frac{1}{8}$	$\frac{1}{16}$
B-Serum E. A. unabs.	A_2	++(+)	+(+)	±	Sp	0
„ „ „	A_3	[±]	[±]	0		
B-Serum E. A. abs.	A_2	++	+	±	0	
m. $\frac{1}{5}$ Vol. A_3 -Blk. ausgeschüttelt aus Serum U.	A_3	[±]	Sp	0		
B-Serum E. A. abs.	A_2	±	0			
m. $\frac{1}{5}$ Vol. unbehandelten A_3 -Blk.	A_3	0	0			

Aus der Tabelle geht hervor, dass die ausgeschüttelten Blk. eine kaum nachweisbare Absorptionsfähigkeit aufweisen, während die unbehandelten deutlich absorbieren.

Untersuchungen über den Erbgang des A_3 -Rezeptors.

Die Familienuntersuchungen umfassen Typenbestimmung von 726 Individuen, von denen 170 A_3 und 33 A_3B sind.

Als Beispiel für die Typenverteilung innerhalb der 26 Familien werden die Stammtafeln Nr. 11, 23 und 26 veröffentlicht.

In Tabelle 10 sind die verschiedenen Elterntypenkombinationen gesammelt, bei denen entweder einer der Eltern A_3 ist oder wo Kinder mit A_3 -Rezeptor vorkommen und der Typus beider Eltern bestimmt werden konnte. Die Sammlung enthält 72 Elternpaare mit 239 Kindern, von denen 82 A_3 und 14 A_3B sind.

In die Tabelle sind, mit * gezeichnet, die Zahlen aus Friedenreichs veröffentlichtem A_3 -Material aufgenommen. Es umfasst 8 Elternpaare mit 50 Kindern, unter ihnen 19 A_3 und 2 A_3B . Von den 6 von Friedenreich veröffentlichten A_3 -Familien sind 2 ausgeschaltet, die Familien H. und J., die später behandelt werden.

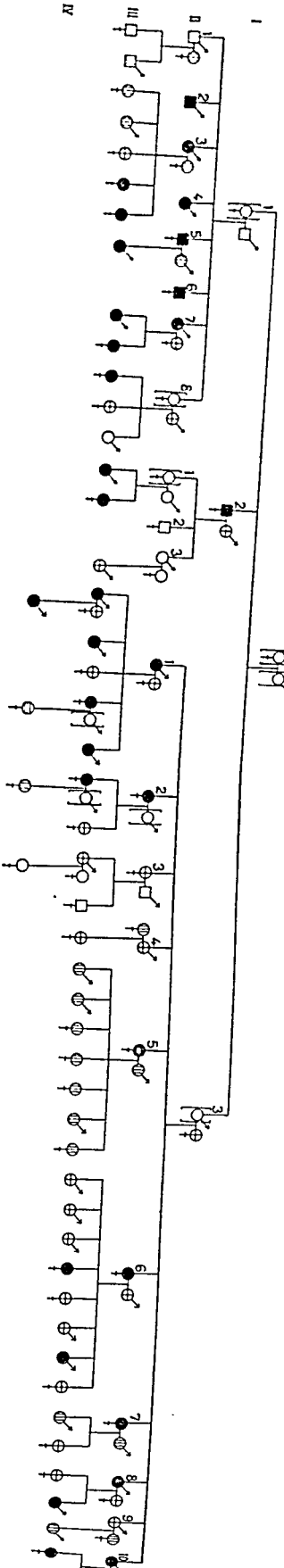
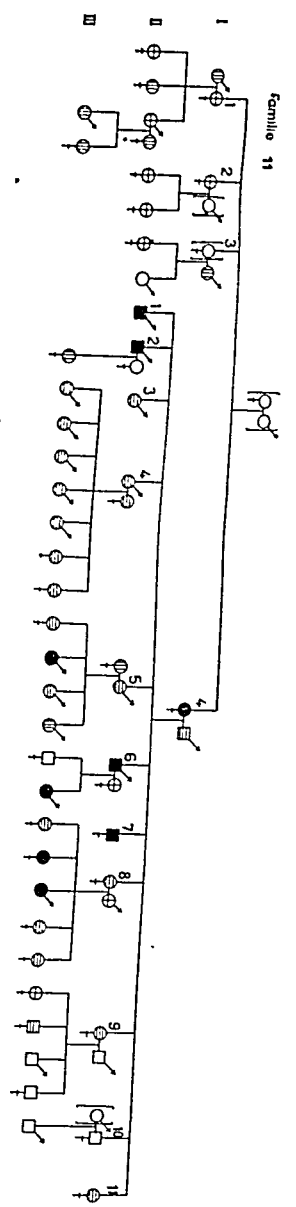
Aus den Stammtafeln und aus Tabelle 10 geht hervor, dass die Vererbungsverhältnisse der A_3 -Eigenschaft vollständig mit den Erfahrungen übereinstimmt, die Friedenreich 1936 veröffentlicht hat und wonach sich der A_3 -Typus auf Basis eines 3. allelomorphen A-Gens entwickelt, das über O dominiert und über das ihrerseits A_1 und A_2 dominieren, ebenso wie A_1 über A_2 . Das 4-Gen-System ist demnach zu einem 5-Gen-System erweitert, das 15 Genotypen und 8 Phänotypen umfasst.

Tabelle 10.

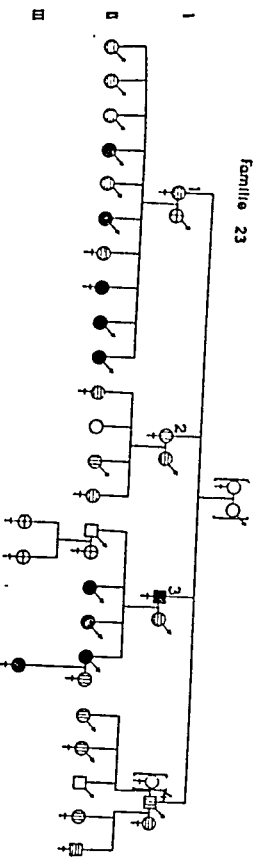
Die mit * bezeichneten Zahlen entstammen Friedenreichs A_3 -Material.

Elternkombinationen	Anzahl Ehen		Anzahl Kinder		Der Bluttypus der Kinder							
					A_1	A_2	A_3	A_1B	A_2B	A_3B	O	
$A_1 \times A_1$	1	1*	5	6*	4	5*	1	1*				
$A_1 \times A_2$	1		4		1		2	1				
$A_1 \times A_3$	17	2*	50	6*	30	4*		11	2*		9	
$A_1 \times A_3B$	4	1*	10	9*	6	1*		2	3*	2	3*	2*
$A_1 \times O$	2	1*	8	4*	4	1*		4	3*			
$A_2 \times A_3$	5		14				8	5			1	
$A_2 \times A_3B$	1		3				1		2			
$A_2 \times O$	3		17				9	8				
$A_3 \times A_1B$	4		11		7					3		1
$A_3 \times A_2B$	3		14				7			5		2
$A_3 \times O$	22	2*	74	20*			42	9*			32	11*
$A_3 \times B$	4	1*	14	5*			2	1*		4	2*	4
$A_3B \times O$	4		9				4					5
$A_3B \times B$	1		6				2			2		2
Zusammen	72	8*	239	50*			82	19*		14	2*	

Familie 26



Familie 23



Zeichen-Erklärung: \oplus Typus O. \otimes Typus A_1 . \ominus Typus A_2 . \bullet Typus A_3 . \square Typus B.
 $---$ A_1B . \boxminus A_2B . \blacksquare A_3B . \circ nicht untersucht [O] tot.

Bei den häufigsten Elternkombinationen mit den zahlreichsten Kindern ist mit Einschluss von Friedenreichs Material das zahlenmässige Verhältnis zwischen den Typen der Kinder folgendes:

Elternkombination	Anzahl Kinder	Anzahl Kinder von jedem Typus		
$A_3 \times O$	94	A_3 : 51	O: 43	O: 9
$A_1 \times A_3$	56	A_3 : 13	A_1 : 34	
$A_2 \times O$	17	A_2 : 9	A_3 : 8	
$A_1 \times O$	12	A_1 : 5	A_3 : 7	

Diese Zahlenverhältnisse stimmen recht genau mit der Verteilung nach der 5-Genhypothese überein, wonach das Verhältnis für die erste Kombination 1 : 1, für die zweite 1 : 2 : 1 sein sollte. Aber das letzte gilt nur, wenn der Genotypus von A_1 ein heterozygotes A_1O ist; da aber Kombinationen mit aufgenommen sind, bei denen die Kinder ausschliesslich A_1 sind und der Genotypus der Eltern deshalb in einer gewissen Anzahl von Fällen A_1A_1 sein kann, muss das Verhältnis, weil ca. 1/7 des A_1 -Typus homozygotes A_1A_1 ist, 1 : 2, 7 : 1 sein. Für die dritte und vierte Kombination soll das Verhältnis 1 : 1 sein. Ferner zeigt die Verteilung der Bluttypen der Kinder Übereinstimmung mit der Hypothese, nach welcher A_1 und A_2 über A_3 dominieren, indem bei der Elternkombination $O \times A_3$ keine Kinder der Typen A_1 oder A_2 vorkommen und in den Typenkombinationen $A_2 \times O$ und $A_1 \times O$ mit A_3 -Kindern keine Kinder des Typus O vorkommen, was daraus zu erklären ist, dass die theoretische Voraussetzung für ein Vorkommen von A_3 in diesen Kombinationen ja sein soll, dass die A-Eltern den Genotypus A_2A_3 , bzw. A_1A_3 aufweisen, also homozygot mit Bezug auf A sind.

Von den Elterntypenkombinationen sind besonders die Kombinationen $A_3 \times A_2B$ und $A_3 \times A_1B$ von Interesse, weil zufolge der Hypothese A_1 , A_2 und A_3 bei den Kindern »ihren Platz getauscht« haben sollen, so dass (nebst B) nur Kinder vom Typus A_1 , bzw. A_2 und A_3B vorkommen. Die Kombination $A_3 \times A_2B$ kommt im ganzen 3-mal vor mit 14 Kindern, von denen 7 A_2 , 5 A_3B und 2 B sind, und die Kombination $A_3 \times A_1B$ kommt 4-mal mit 11 Kindern im ganzen vor, von denen 7 A_1 , 3 A_3B und 1 B sind. Die A_1 - und A_2 -Kinder dieser Kombination können entweder den Genotypus A_1A_3 oder A_1O haben, bzw. A_2A_3 oder A_2O , und wo noch eine Generation untersucht werden konnte, ist dies bestätigt worden, indem A_3 - und O-Kinder nicht gleichzeitig angetroffen wurden (siehe Familie 11).

Eine besonders interessante Bluttypenkombination wurde in der Familie 23 festgestellt. Die 4 Individuen der 1. Generation sind als die Typen A_2 , A_1 , A_3B und A_1B bestimmt worden. Diese 4 Typen kön-

nen nur durch eine einzige Kombination bei den Eltern entstanden sein, nämlich $A_1A_3 \times A_2B$, und gleichzeitig treten alle 4 möglichen Kombinationen auf. Dem Erbgang nach soll der Genotypus bei diesen A_1 - und A_2 -Individuen A_1A_2 bzw. A_2A_3 sein. Dies bestätigt sich auch, indem das A_2 -Individuum, mit einem Individuum des Typus O verheiratet, 10 Kinder hat, von denen 5 A_3 und 5 A_2 sind. Das A_1 -Individuum ist mit einem Individuum des Typus A_2 verheiratet, und deshalb kann nicht nachgewiesen werden, dass der Genotypus A_1A_2 ist, aber andererseits ist keines der 3 Kinder vom Typus O. Ferner ist zu bemerken, dass nach den Genotypen der Individuen der ersten Generation kein Individuum der zweiten Generation den Typus O aufweisen darf, und dies wird auch durch die Familienuntersuchung bestätigt.

Familie H. (Friedenreich).

Wie schon in der Einleitung erwähnt, findet sich in Friedenreichs A_3 -Material eine Ausnahme von der Erbgangregel, indem ein Mann, der nach der Typenverteilung der Eltern genotypisch A_2A_3 sein soll, einen Sohn hat, der den Typ O darstellt, nach der Alleltheorie eine Unmöglichkeit. Die Mutter ist A_2 . Nach dem MN-System steht der Vaterschaft des Mannes nichts im Wege.

Wie von Friedenreich angeführt, kann diese Abweichung natürlich durch Illegitimität erklärt werden, aber es findet sich in dieser Familie auch eine andere Eigentümlichkeit, indem die Blk. aller Mitglieder dieser Familie, die von Friedenreich als Typus » A_2 « bezeichnet wurden, nicht wie typische A_2 -Blk. reagieren, sondern neben einer normalen α_2 -Reaktion (von derselben Stärke wie Standard- A_2 -Blk.) auch eine schwache α_1 -Reaktion aufweisen. Friedenreich stellt die Hypothese auf, dass die » A_2 «- und A_3 -Typen dieser Familie nicht auf allelen Genen beruhen, sondern auf die Einwirkung eines komplementären rezeptormodifizierenden, in casu abschwächenden Gens auf das A_1 - bzw. A_2 -Gen zurückzuführen sind, so dass A_1 + dem modifizierenden Gen phänotypisch als das obengenannte A_2 erscheint und A_2 + dem modifizierenden Gen als A_3 . Hierdurch erklärt sich auch die Abweichung von der Alleltheorie, indem der erwähnte Mann genotypisch nicht A_2A_3 ist, sondern A_1O + dem modifizierenden Gen und deshalb imstande, mit einer Frau des Typus A_2 (Genotypus A_2O) sowohl O- als » A_2 «- und A_3 -Kinder zu bekommen. Infolge dieser Theorie soll also der A_3 -Typus durch Einwirken eines rezeptormodifizierenden Gens auf die normale A_2 -Anlage hervorgebracht werden können.

Ich habe an einigen Mitgliedern der Familie eine nochmalige Untersuchung vorgenommen und dabei das von Friedenreich Angeführte vollständig bestätigt gefunden.

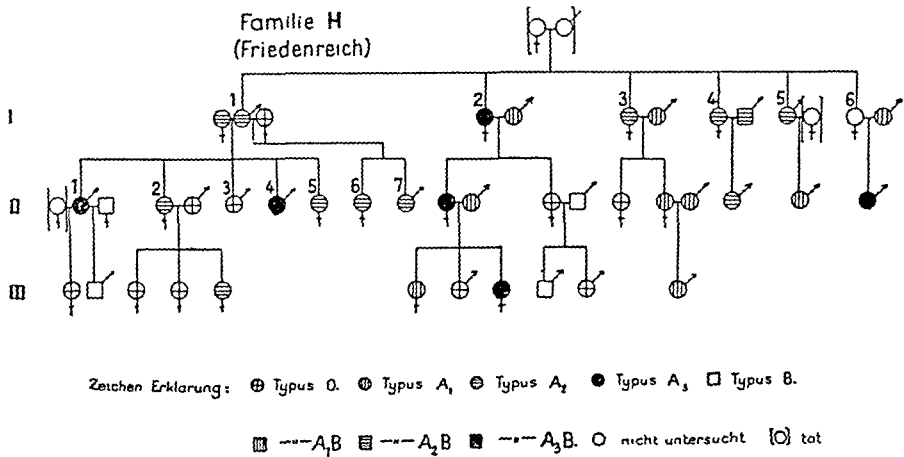
Die Typenverteilung der Familie ist aus dem Stammbaum Familie H. (Friedenreich) ersichtlich.

Friedenreichs Annahme zur Erklärung der beobachteten Unregelmässigkeit in der Familie H. scheint mir einleuchtend, weil dadurch sowohl die Abweichung von der Alleltheorie als auch die atypischen A_2 -Reaktionen sich erklären lassen. Gegen eine Erklärung durch Illegitimität spricht eine ausgesprochene Ähnlichkeit zwischen Vater und Sohn.

Die Typeneigentümlichkeiten der Familie H. sind als einmaliges Phäno-

men aufzufassen, da die oben beschriebenen 26 A_3 -Familien zeigen, dass sich der A_3 -Typus normal auf Basis eines 3. allelomorphen A-Gens entwickelt.

Die Einwirkung komplementärer Gene auf das Blutgruppensystem ist keineswegs eine unbekannte Erscheinung, indem die ganze »Ausscheider-Nicht-Ausscheider«-Theorie ein Beispiel für modifizierende Gene darstellt. Ferner wird unten ein Bluttypus A_x beschrieben werden, wo die Annahme eines Einwirkens komplementärer Gene auf das alkohollösliche Antigen-system die beobachteten serologischen Verhältnisse erklären kann.



Friedenreich hat ausserdem in einer Familie einen schwachen N-Rezeptor nachgewiesen (noch nicht veröffentlicht), der serologisch von N_2 (Crome (1), Friedenreich (7)) unzertrennbar ist und wo sich der Erbgang nicht durch Annahme eines Allelgens erklären lässt, so wie es bei N_2 der Fall ist.

Untersuchungen über den Antigengehalt des Speichels von A_1 -, A_2 - und A_3 -Individuen.

Zum Nachweis eines eventuell schwächeren Antigengehaltes im Speichel von A_3 -Individuen als in dem von A_1 - und A_2 -Personen wurde der Speichel von 72 A_1 -, 53 A_2 - und 25 A_3 -Individuen untersucht. Die Untersuchung geschah durch Agglutinationshemmung. In einer Reihe von Reagensgläsern wird 0,1 cm³ Speichel in den Konzentrationen $\frac{1}{1}$, $\frac{1}{2}$, $\frac{1}{4}$, $\frac{1}{8}$, $\frac{1}{16}$ etc. vermisst. Jedem Gläschen wird 0,1 cm³ Anti-A-Isoserum zugesetzt, und zwar so verdünnt, dass es mit den A_1 -Blk. gerade die maximale Agglutination ergibt. Hierdurch wird bei Verdünnung mit gleichen Teilen Antigenlösung eine Agglutination von der Stärke +++ erzielt. Eine solche Bemessung der Serumstärke bewirkt, dass selbst kleine Antigenmengen, die nur eine Abnahme der Agglutinationsstärke ergeben, erkennbar sind. Die Mischung Antigen-Antistoff lässt man bei Zimmertemperatur eine Stunde stehen. Darauf wird 1 Tropfen 5 %-iger Blk.suspension zugetzt, und 3 Stunden später wird mit blossen Auge abgelesen. Das A-Antigen des Speichels bindet den Antistoff des Serums und bewirkt ein Ausbleiben der Agglutination. Die Anzahl Gläsern, in denen keine Agglutination vorkommt, ist ein Ausdruck für die Antigenmenge des Speichels.

Tabelle 11.

		Agglutinationshemmungstitern																		
		0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
Anzahl Speichelproben von Individ. des Typus:	A ₁	17	8	0	0	0	0	0	2	9	6	6	5	2	7	3	3	2	2	0
	A ₂	7	4	1	0	0	0	6	11	10	5	2	4	0	3	0	0	0	0	0
	A ₃	5	1	0	0	1	1	4	7	2	2	2	0	0	0	0	0	0	0	0

Nach Zusatz des Serums sind die Speichelkonzentrationen in den Gläschen $\frac{1}{2}$, $\frac{1}{4}$, $\frac{1}{8}$, $\frac{1}{16}$ u. s. w. oder $\frac{1}{2^1}$, $\frac{1}{2^2}$, $\frac{1}{2^3}$ u. s. w. bis $\frac{1}{2^n}$ n, das auch die Anzahl der Gläschen angibt, wird im folgenden als Ausdruck für die Antigenkonzentration angewendet. Zu jeder Versuchsreihe wird eine Speichelprobe von ♀ L., Typus A₁ herangezogen, weil sich bei diesem Individuum der Antigengehalt des Speichels seit Jahren als recht konstant erwiesen hat und diese konstante Hemmung Gewähr leistet, dass man immer unter denselben Versuchsbedingungen arbeitet.

Alle Speichelproben wurden kurz nach der Entnahme 20 Minuten gekocht, um die spontane Zersetzung des Typenantigens zu verhindern.

Die durch Agglutinationshemmungsversuche mit dem Speichel von A₁, A₂- und A₃-Individuen gefundenen Antigenkonzentrationen sind in der Tabelle 11 angegeben.

Der Speichel von A₁-, A₂- sowie A₃-Individuen zeigt einen Antigengehalt, der sich klar auf 2 Gruppen verteilt, »Ausscheider« und »Nicht-Ausscheider« (Schiff und Sasaki (31)), mit einem deutlichen Intervall zwischen den beiden Gruppen. In der Nicht-Ausscheidergruppe wird, wie auch von G. Hartmann (17, 18) angegeben, in mehreren Fällen ein geringer Antigengehalt nachgewiesen. Durchschnittlich zeigt der A₁-Speichel einen grösseren Antigengehalt als A₂, und A₂ wiederum einen grösseren Antigengehalt als A₃. Aus der Tabelle 11 geht hervor, dass das Intervall zwischen der Ausscheider- und der Nicht-Ausscheidergruppe von A₁ über A₂ nach A₃ abnimmt, so dass sich bei schwächeren A-Typen eine Neigung zur Verschmelzung der beiden Gruppen bemerkbar macht, was mit den Beobachtungen Hartmanns bei Bestimmung des wasserlöslichen Antigens in Organen verglichen werden kann, wobei es sich zeigte, dass, je niedrigeren Antigengehalt die Organe hatten, es umso schwieriger war, die Ausscheider- und Nicht-Ausscheidergruppen zu trennen, da die beiden Gruppen in einander übergriffen.

Zusammenfassung.

1) Es werden die serologischen Kennzeichen des Typus A₃ beschreiben, wovon im ganzen 203 (170 A₃ und 33 A₃B) nachgewiesen worden sind. Charakteristisch ist die eigentümliche Agglutination, eine Mischung nichtagglutinierten und agglutinierten Blk. in allen Iso-B- und Anti-A-Immunsereen. Es wird gezeigt, dass diese Agglutination auf einer ungleichmässigen Stärke des A-Rezeptors der A₃-Blk. beruht. Die Absorptionsfähigkeit liegt zwischen A₂ und A₂B. Im Serum von 60 A₃-Individuen wird in keinem Falle bei Zimmertemperatur irreguläres α_1 nachgewiesen.

In Anti-O- h ltigem Ochsen Serum werden A_3 -Blk. kr ftiger agglutiniert als A_2 -Blk.

Der A_3 -Typus betr gt ungef hr 1 ‰ der A-Gruppe.

Durch eine Untersuchung von 26 A_3 -Familien wird wahrscheinlich gemacht, dass sich A_3 auf Basis eines 3. allelomorphen A-Gens vererbt.

Im Speichel von A_3 -Individuen, die Ausscheider sind, wird weniger A-Antigen nachgewiesen als bei Ausscheidern des A_1 - und A_2 -Typus.

(Die Literaturliste findet man in einer Fortsetzung dieses Artikels, die unter dem Titel: Serologische und genetische Untersuchungen  ber seltene A-Typen

II. Die Bluttypen A_4 , A_5 und A_x in dieser Zeitschrift erscheinen wird.)

SEROLOGISCHE UND GENETISCHE UNTERSUCHUNGEN ÜBER SELTENE A-TYPEN BEI MENSCHEN

II. DIE BLUTTYPEN A_4 , A_5 UND A_x .

Von *Arne Gammelgaard*.

(Eingegangen bei der Redaktion am 29. Mai 1943.)

Ich habe in einem vorausgehenden Artikel in dieser Zeitschrift meine Untersuchungen über den Bluttypus A_3 mitgeteilt. Was Berichte über Untersuchungen auf dem hier vorliegenden Gebiete betrifft, verweise ich auf diesen Artikel, wo auch die Beschaffung und Einsammlung des Materials ausführlich besprochen wird.

Der Bluttypus A_4 .

Das Material umfasst 2 Fälle, der eine wurde aus den 52.000 Typenbestimmungen am Staatlichen Seruminstitut und der andere aus ungefähr 10.000 Typenbestimmungen im Institut für Gerichtliche Medizin herausgeholt. Es wurden Typenbestimmungen an Familienmitgliedern der beiden Individuen vorgenommen (und zwar an Venenblut, ca. 24 Stunden nach der Entnahme der Blutprobe), und dabei wurden im ganzen 10 Individuen des Typus A_4 , keines vom Typus A_4B festgestellt.

Serologisch ist A_4 vor allem durch eine schwache Agglutination in Iso-B-Seren gekennzeichnet, eine Agglutination, die schwächer ist als A_2B und die in keinem der angewendeten Seren (sämtlichen, in denen A_3 untersucht wurde) das für A_3 -Blk. charakteristische Agglutinationsbild aufweist. Tabelle 1 zeigt die Agglutinationsstärke von A_2 -, A_2B -, A_3 - und A_4 -Blk. in 5 Iso-B-Seren und 1 Anti-A-Immuns serum.

Es erhellt aus Tabelle 1, dass der früher erwähnte eigentümliche Unterschied, der in der Agglutinationsstärke zwischen A_3 und A_2B auftritt, sich auch entsprechend zwischen A_3 und A_4 geltend macht, d. h. in den starken Seren agglutiniert A_4 anscheinend stärker als

Tabelle 1.

Die A₂-, A₂B-, A₃- und A₄-Blk. Agglutination in 5 Iso-B-Serum und in 1 Anti-A-Immunserum.

Blutk.	Iso-B-Seren					Immunserum
	J. (1024)	U. (512)	Johs. (128)	E. A. (32)	M. (16)	K. 19 (512)
A ₂ (H.).....	++++	++++	++++	+++	++	++++
A ₂ B (Kr.)	++++	++++	++(+)	+	Sp	++++
A ₃ (W.)	[+ (+)]	[+ (+)]	[+]	[±]	[±]	[±]
A ₄ (Else).....	++++	+++	++	Sp	0	++++

A₃, während in den schwachen Seren A₃ noch agglutiniert, aber A₄ überhaupt nicht. In Immunseren ist der Unterschied ebenso wie in Isoseren, nur noch ausgesprochener, indem A₃ bezeichnenderweise nur sehr schwach, A₄ dagegen maximal agglutiniert.

A₄ verhält sich somit in den verschiedenen Seren, was die Intensität der Agglutination betrifft, ebenso wie A₂B, nur ist sie ca. + niedriger, und deshalb ist bei der qualitativen Agglutinationsuntersuchung eine Untersuchung von A₂ und A₄ leichter in den schwächeren Iso-B-Seren, wo der Unterschied der Agglutinationsstärke in die Augen springt, während er sich in den starken Iso-B-Seren und in den Anti-A-Immunseren mehr verwischt. Der grosse Unterschied der Agglutination zwischen A₃ und A₄ in Anti-A-Immunseren wurde bei sämtlichen Immunseren nachgewiesen, in denen A₃, wie früher erwähnt, untersucht worden ist.

Durch eine quantitative Agglutinationsuntersuchung zeigt es sich, dass A₄ ca. 3 Stufen unter A₂ liegt, siehe Tabelle 2.

Tabelle 2.

Austitrierung von Iso-B-Serum V. gegenüber A₂-, A₂B-, A₃- u. A₄-Blk.

Blutk.	1/1	1/2	1/4	1/8	1/16	1/32	1/64	1/128
A ₂ (H.)	++++	++++	+++	+++	++	+	±	0
A ₂ B (Kr.)	++++	+++	++	+(+)	±	0		
A ₃ (W.)	[+ (+)]	[+]	[+]	[±]	[±]	Sp	0	
A ₄ (Else)	+++	++	+(+)	±	0			

Diese quantitative Agglutinationsuntersuchung ist in mehreren der früher erwähnten ausgesuchten Iso-B-Seren vorgenommen worden. Dabei hat sich gezeigt, dass der Titerunterschied zwischen A₂ und A₄ recht konstant war. Eine ähnliche Variation der Titerunterschiede,

wie sie Friedenreich für A_1 und A_2 in verschiedenen B-Seren nachgewiesen hat, wurde bei A_2 und A_4 nicht beobachtet, was darauf schliessen lässt, dass A_4 zu sämtlichen Fraktionen von Anti-A eine konstante quantitativ schwächere Affinität aufweist als A_2 .

Dass die Rezeptorstärke von A_4 schwächer ist als von A_2 B, kann nicht nur durch eine Agglutinations-, sondern auch durch Absorptionsuntersuchung nachgewiesen werden. Tabelle 3 zeigt die Absorptionsfähigkeit von A_4 , verglichen mit A_2 und A_2 B.

Tabelle 3.
Iso-B-Serum Johs. abs. mit $\frac{1}{5}$ Vol. A_2 -, A_2 B- und A_4 -Blk.
Serum autitriert gegenüber A_2 - und A_4 -Blk.

	Blk.	$\frac{1}{1}$	$\frac{1}{2}$	$\frac{1}{4}$	$\frac{1}{8}$	$\frac{1}{16}$	$\frac{1}{32}$
B-Serum Johs. unabs.	A_2	++++	+++	+(+)	+	\pm	0
	A_4	++	+	+	\pm	0	
B-Serum Johs. abs. m. $\frac{1}{5}$ Vol.) A_2 -Blk. (H.)	A_2	\pm	0				
	A_4	0	0				
B-Serum Johs. abs. m. $\frac{1}{5}$ Vol.) A_2 B-Blk. (Kr.)	A_2	++	+(+)	\pm	0		
	A_4	\pm	0				
B-Serum Johs. abs. m. $\frac{1}{5}$ Vol.) A_4 -Blk. (Knud)	A_2	++	++	+	\pm	0	
	A_4	\pm	\pm	0			

Aus der Tabelle 3 geht ferner hervor, dass weder durch die Absorption mit A_2 B noch mit A_4 eine elektive Verschiebung des Titerunterschiedes zwischen A_2 und A_4 eingetreten ist, dieser hält sich vielmehr fast konstant, und die durch Absorption von Serum eintretende Schwächung entspricht einer einfachen Verdünnung, d. h. es lässt sich keine Fraktion von Anti-A nachweisen, die gleich α_1 weniger avid wäre und deshalb so wie α_1 eine verhältnismässig elektive Affinität zu den mit kräftigerem Rezeptor ausgestatteten Blk., in casu den A_2 -Blk., aufwiese.

Bei Untersuchung in Anti-O-hältigem Ochsen Serum agglutinierten die Blk. der 10 A_4 -Individuen etwas kräftiger als Standard A_2 -Blk., eher wie A_3 -Blk.

Ein Individuum, Familie 2, I. Generation, Nr. 1 muss genotypisch als A_2A_4 bezeichnet werden. Die Blk. dieses Individuums agglutinierten auch etwas kräftiger in Anti-O-hältigem Ochsen Serum als Standard A_2 -Blk.

Von den 10 A_4 -Individuen zeigten 4 im Serum irreguläres α_1 , das sich bei Zimmertemperatur nachweisen liess (Agglutinationsstärke mit A_1 -Blk.: \pm , \pm , ++, \pm). Ferner enthielt ein Serum irreguläres α_1 , das bei 4° nachweisbar war (Titer 8). Es wurde keine Beziehung zwischen dem Gehalt des Serums an irregulärem α_1 und

Anti-B nachgewiesen. Im Serum von A₄-Individuen tritt somit im Gegensatz zum Serum von A₃-Individuen irreguläres α_1 prozentuell recht häufig auf, und die Vermutung Friedenreichs und Waalers, dass das Auftreten des irregulären Agglutinins durch Schwäche des A-Rezeptors begünstigt wird, scheint sich demnach zu bestätigen.

Die Familie J. (Friedenreich).

Friedenreich behandelt, wie schon früher erwähnt, anlässlich der Variation des A₃-Rezeptors eine Familie, deren A₃-Mitglieder eine von den übrigen A₃-ern abweichende Absorption und Agglutination aufwiesen. Ein Mitglied dieser Familie (♂ J.) war zur Blutgruppenbestimmung im Institut für Gerichtliche Medizin gewesen, da er 1939 in der Paternitätsklage 951/39 als alleiniger Erzeuger benannt worden war. Die Mutter war A₁, das 6 Monate alte Kind war »schwaches A«. Sowohl bei dieser Untersuchung als auch bei mehreren Kontrolluntersuchungen, die sich über 1 Jahr erstreckten, ergab es sich, dass die Blk. des ♂ J. sowohl bei Agglutinationsuntersuchung als auch bei Absorptionsversuchen sich vollständig ebenso verhielten wie bei dem obenbeschriebenen A₄, womit direkte Vergleichen wiederholt vorgenommen wurden. Friedenreich hat mir gütigst sein Protokoll über die Typenbestimmung der Familie J. zur Verfügung gestellt, und beim Durchgang desselben zeigt es sich, dass die Blk. der als A₃ bezeichneten Mitglieder in ihrem Agglutinations- und Absorptionsverhalten vollkommen mit A₄ übereinstimmen. Friedenreich hat nur in einem einzigen Fall das Serum untersucht, und zwar bei einem Bruder des ♂ J., und dabei fand er ein kräftiges irreguläres α_1 , das bei 10° wirkte. Im Serum des ♂ J. fand ich ein irreguläres α_1 , mit einem Titer von 8 bei 4°, aber ohne Wirkung bei Zimmertemperatur. Bei Untersuchung der Blk. des Kindes (Knabe C.) ergab sich ein A-Rezeptorgehalt, noch schwächer als beim Vater, wie es aus der Tabelle 4 hervorgeht.

Tabelle 4.

Agglutination der Blk. von A₂, A₂B, A₄ (Vater) und A₄ (6 Monate alter Sohn) in 6 Iso-B-Seren und 2 Anti-Immunseren.

Blutk.	B-Sera						Immunsera	
	J. (1024)	U. (512)	V. (256)	Johs. (128)	E. A. (32)	M. (16)	K. 19 (512)	G. 1 (256)
A ₂ (H.)..	+++++	+++++	+++++	+++++	+++	++	+++++	+++++
A ₂ B (Kr.)	+++++	+++++	+++	+++	+	Sp	+++++	+++++
♂ J.	+++	+++	++	+(+)	Sp	0	+++++	+++
Knabe C.	+(+)	+(+)	±	Sp	0	0	+++	++

Die Blk. des Kindes C. wurden 15 Monate später, als das Kind $1\frac{3}{4}$ Jahre alt war, wiederum untersucht, und zum Vergleich war eine Blutprobe des ♂ J. beschafft worden. Verglichen mit der früheren Untersuchung zeigte es sich, dass die Blk. des Kindes jetzt einen etwas kräftigeren A-Rezeptor hatten, der aber doch noch immer etwas schwächer war als der des Vaters. Der Unterschied betrug jetzt bei der Untersuchung der Agglutination ca. \pm gegen ca. $+$ (+) bei der ersten Untersuchung.

Ein ähnliches Verhalten fand ich bei der Untersuchung von Vater A_4 und 2-jährigem Kind A_4 aus der Familie 2. Das Kind zeigte auch hier einen etwas schwächeren A-Rezeptor als der Vater.

Die Untersuchung der 2 Kinder von 2 bzw. $1\frac{3}{4}$ Jahren scheint zu zeigen, dass der A-Rezeptor erst spät seine volle Entwicklung erreicht.

Die » A_3 -Mitglieder« der Familie J. und die 10 A_4 , die den 2 von mir untersuchten Familien angehören, müssen demnach zweifellos denselben Typus darstellen, der sich sowohl von A_2 als auch von A_3 klar unterscheidet.

Die Unterscheidung von A_3 ist nicht schwierig, da A_4 keine Tendenz zeigt, in dem für A_3 charakteristischen Bild zu agglutinieren und deshalb schon durch eine qualitative Agglutinationsuntersuchung in Iso-B-Seren, wo der Kontrast in den starken Seren besonders deutlich ist, von A_3 unterschieden werden kann. In Immun-Anti-A-Seren tritt der Unterschied noch deutlicher hervor.

Von A_2 kann A_4 nur durch seine quantitativ schwächere Rezeptorstärke unterschieden werden, und massgebend für die A_4 -Bestimmung muss deshalb die Angabe sein, wie schwach die Rezeptorstärke im Verhältnis zu A_2 ist, und hier kann A_2B benutzt werden, da A_4 ausnahmslos eine schwächere Rezeptorstärke aufgewiesen hat als der A_2 -Rezeptor in A_2B . Dieser Unterschied der Rezeptorstärke zwischen A_2 und A_4 erscheint sowohl bei der qualitativen Agglutinationsuntersuchung auf dem Objektträger (wo er doch in den starken Iso-B-Seren und in Anti-A-Immunseren verschwimmt) wie auch bei der quantitativen Bestimmung der Rezeptorstärke durch Austitrierung und durch Absorptionsuntersuchung.

Über die Häufigkeit des Auftretens eines A_4 -Rezeptors können die hier besprochenen 3 Fälle nichts anzeigen, da die Möglichkeit, A_4 zu übersehen, sehr gross ist. Teils kann es als A_2 , teils als O bestimmt werden. Für O kann A_4 angesehen werden, wenn man so schwache Seren anwendet, dass die Agglutination mit A_2B schwer zu erkennen ist, da die Serumuntersuchung, wegen des in den meisten Fällen vorgefundenen Gehaltes an irregulärem α_1 keine Kontrolle bietet, wenn nur A_1 -Testblk. angewendet werden.

Nr. 3, I. Generation, Typus A_4 , ist mit einem Typus A_1 verheiratet, dessen Genotypus A_1O sein muss. In der Ehe sind 9 Kinder, nämlich 5 A_1 , 2 A_4 und 2 O . Durch eines der Kinder, Nr. 7, II. Generation Typus A_4 , mit Typus O verheiratet, vererbt sich die Eigenschaft weiter in die III. Generation.

In der Familie 1 hat Nr. 1, I. Generation Typus A_1 , 2 A_4 -Kinder, und Nr. 4, I. Generation, 1 A_4 -Kind.

In der Familie 2 hat Nr. 1, I. Generation Typus A_2 , in der Ehe mit Typus O 6 A_4 -Kinder, von denen Nr. 3, II. Generation, in seiner Ehe mit Typus O die Eigenschaft weiter in die III. Generation führt.

In der Elternkombination $A_4 \times O$ kommen somit nur A_4 - und O -Kinder vor, aber keine Kinder mit »höherem A « (A_1 oder A_2).

Den angeführten Typenverteilungen innerhalb der Familien zufolge beruht der Erbgang des A_4 -Rezeptors zweifellos auf einem selbständigen A -Gen (A_4), das mit den übrigen Bluttypengenen allelomorph ist, und das über O dominiert und seinerseits von A_1 und A_2 dominiert wird, auf dieselbe Weise, wie A_1 über A_2 dominiert.

Bestimmung der A-Antigenmenge im Speichel von A_4 -Individuen.

Die Bestimmungen sind durch Agglutinationshemmungsversuche nach dem unter A_3 angegebenen Verfahren vorgenommen worden.¹⁾ Die Speichelproben wurden in den allermeisten Fällen kurz nach der Entnahme gekocht. Einzelne wurden mir in sterilen W.R.-Gläsern übersandt, auf deren Boden ein Fluroidsalz eingetrocknet war, um bakterielle Enzymwirkung zu verhindern. Wenn ich um die Proben bat, habe ich die Betreffenden (es gilt die J.-Familie) dringend gebeten, die Proben möglichst bald nach der Entnahme abzusenden, so dass allerhöchstens 24 Stunden bis zur Vornahme der Bestimmung vergingen. Einige Kontrollversuche mit Speichel, der auf dieselbe Weise entnommen war und bei Zimmertemperatur 24 Stunden stehen gelassen worden war, haben eine kaum nachweisbare Abnahme der Rezeptorstärke gezeigt.

Die Untersuchung umfasst 9 Speichelproben von A_4 -Individuen der Familie J. (alle übersandt), 3 der Familie 1 und 7 der Familie 2 (alle ohne Ausnahme gekocht). Der Hemmungstiter n dieser 19 Proben verteilt sich wie folgt:

Hemmungstiter n	0	1	2	3	4
Anzahl Speichelproben von Individuen des Typus A_4 ..	16	1	1	1	0

Bei der Agglutinationshemmung zeigen alle Speichelproben einen so geringen A -Gehalt, dass sie in die Nicht-Ausscheidergruppe der bisher bekannten A -Typen fallen. Dies lässt sich jedoch kaum damit erklären, dass sie alle homozygot das rezessive Gen »s« zur Nicht-Ausscheidung enthielten, da mehrere A_1 - oder A_2 -Geschwister und -Eltern von A_4 -Individuen auch auf den Antigengehalt des Speichels

¹⁾ Acta path. et microbiol. Scand. 21, 535, 1944.

untersucht wurden und sich alle dabei als Ausscheider erwiesen. Liesse man sich endlich zu der unwahrscheinlichen Annahme verleiten, dass alle untersuchten Speichelpuben enzymatisch zersetzt gewesen seien, könnte es höchstens die 9 Fälle erklären, da die übrigen Speichelpuben kurz nach der Entnahme gekocht wurden, wodurch eine spontane Zersetzung der Rezeptorsubstanz verhindert wird.

Die geringe Rezeptorstärke des Speichels kann eher mit dem schwachen Blk.-Rezeptor in Verbindung gebracht werden und darauf zurückzuführen sein, dass das komplementäre Gen für die Ausscheidung auf Basis des mit geringer Rezeptorstärke ausgestatteten Bluttypengens A_4 eine so spärliche Rezeptormenge in den Organen entwickelt, dass nur eine Spur des Rezeptors nachgewiesen werden kann und deshalb eine Unterscheidung zwischen Ausscheider und Nicht-Ausscheider unmöglich ist.

Der Bluttypus A_5 .

Die folgenden Untersuchungen sind im wesentlichen 1940 veröffentlicht worden: Gammelgaard und Marcussen: Nachweis eines vierten allelomorphen A-Gens (A_4). Ztschr. f. Immunitätsf. u. exper. Therap. 98: 411, 1940, worauf ich verweise und wozu ich hier nur einige Ergänzungen bringen werde.

Nach den oben angeführten Untersuchungen muss der früher als A_4 bezeichnete A-Rezeptor nunmehr als A_5 in die A-Reihe eingegliedert werden, da es ein merkbar schwächerer A-Rezeptor ist als der oben beschriebene.

Seit 1940 habe ich in 2 Paternitätsklagen weitere 3 Personen des Typus A_5 gefunden, deren Familien ich zur Beleuchtung des Erbganges noch nicht habe untersuchen können. Diese 3 A_5 -Rezeptoren sind alle mit Blk. von einem der in der Ztschr. f. Immunitätsf. 1940 als A_4 bezeichneten Individuen verglichen worden.

Im ganzen sind so 24 A_5 - und 3 A_5B -Individuen untersucht worden, und es wird von Interesse sein, die Agglutinationsstärke der A_3 -, A_4 - und A_5 -Blk. zu vergleichen.

Aus Tabelle 5 geht hervor, dass A_5 deutlich schwächer ist als A_4 und bedeutend schwächer als A_2 . Das Agglutinationsbild der A_5 -Blk. zeigt keine Tendenz nach der für A_3 charakteristischen Richtung.

Es ist ein A_5B mit aufgenommen, und man sieht, dass B eine stark hemmende Wirkung auf A_5 ausübt, so dass A_5B nur in einem B-Serum mit dem Titer 1000 erkennbar ist.

Die Absorptionsfähigkeit des A_5 -Rezeptors ist, wie im früheren Artikel erwähnt, sehr gering. In Anti-O-hältigem Serum agglutinieren A_5 -Blk. ebenso stark wie Standard A_2 -Blk. Im Serum von 15 A_5 -Individuen wurde in 13 Fällen irreguläres α_1 nachgewiesen, das bei Zimmertemperatur wirksam war. In 2 Fällen war das irreguläre Anti-A

Tabelle 5.

Agglutination von A_2 -, A_2B -, A_3 -, A_4 -, A_5 - und A_5B -Blk. in Iso-B-Seren und Anti-A-Immunseren.

Blutk.	B-Seren						Immunseren	
	J. (1024)	U. (512)	V. (256)	Johs. (128)	E. A. (32)	M. (16)	K. 19 (512)	G. 1 (256)
A_2 (H.).....	++++	++++	++++	++++	+++	++	++++	++++
A_2B (Kr.)...	++++	++++	++++	+++	+	Sp	++++	++++
A_3 (W.).....	[+(+)]	[+(+)]	[+]	[+]	[±]	[±]	[±]	[±]
A_4 (J.).....	++++	++++(+)	+++	++	Sp	0	++++	+++
A_5 (Clara)...	+++	++	+(+)	±	0	0	+++	+(+)
A_5B (Edith)...	+	±	0	0	0	0	+(+)	±

so kräftig, dass es auch A_2 -Blk. bei Zimmertemperatur agglutinierte. Dieser Befund spricht noch weiter für die Theorie von Friedenreich und Waaler.

Betreffs Untersuchungen über den Erbgang wird auf den früheren Artikel verwiesen.

Mit Bezug auf die Diagnostizierung von A_5 gilt dasselbe wie für den Typus A_4 , dass keine spezifiken Reaktionen nachgewiesen wurden. A_5 unterscheidet sich nach den angewendeten serologischen Untersuchungsmethoden von A_2 und A_4 durch einen quantitativ noch schwächeren A-Rezeptor als A_4 , und sein Charakteristikum muss sein, dass er bedeutend schwächer ist als der A-Rezeptor in A_2B , während A_4 nur um eine Kleinigkeit schwächer ist.

A_5 habe ich, wie erwähnt, 1 Mal bei ca. 60.000 und jetzt später 3 Mal bei ca. 5000 Blutgruppenbestimmungen nachgewiesen. Diese Zahlen verraten natürlich nichts über die Häufigkeit des Auftretens eines A_5 -Rezeptors, aber die zuletzt nachgewiesenen Fälle sprechen dafür, dass A_5 bedeutend häufiger vorkommt als 1 : 60000, wenn man nur dem Vorhandensein eines so schwachen A-Rezeptors seine Aufmerksamkeit schenkt. Wo kein A_5 bestimmt wird, wird er sicher als Typus O rubriziert werden, wozu gerade das häufige Auftreten von irregulärem α_1 im Serum beiträgt.

Bestimmung der Antigenmenge im Speichel von A_5 -Individuen.

Die Bestimmung ist durch Agglutinationshemmungsversuche nach dem früher angeführten Verfahren vorgenommen worden. Alle Proben werden kurz nach der Entnahme 20 Minuten gekocht und ungefähr 24 Stunden später untersucht. Der Hemmungstiter n verteilt sich bei 18 Proben auf folgende Weise:

Hemmungstiter n	Anzahl Speichelproben von Individuen des Typus:	
	A ₅	A ₅ B
0	13	3
1	2	

Alle Speichelproben weisen so wie bei A₄ so geringe Hemmung aus, dass sie in die Nicht-Ausscheidergruppe fallen würden, wenn es sich nicht um Individuen des A₁-, A₂- oder A₃-Typus handelte. Dass der geringe Antigengehalt der Speichelproben darauf beruhen sollte, dass alle diese Individuen homozygot das Gen der Nicht-Ausscheidereigenschaft »s« besäßen, kann in diesen Fällen noch weniger als Erklärung gelten; denn keiner von den 3 A₅B weist im Iso-B-Serum eine Agglutinationshemmung auf, aber 2 von ihnen hemmen die Agglutination im Iso-A-Serum, und zwar mit dem Titer (dem exponentiellen Titer n) 8 bzw. 10, während der 3. auch im Iso-A-Serum keine Hemmung zeigt. Das heisst also, dass die 2 Ausscheider sind, weil die Ausscheidereigenschaft nicht an eine einzelne Bluttypeneigenschaft gebunden ist, sondern im AB-Typus ebenso sehr für den A- wie für den B-Rezeptor gilt. Der 3. dagegen ist Nicht-Ausscheider im eigentlichen Sinn, da auch kein B-Rezeptor nachgewiesen werden kann. Die A₅B-Ausscheider sind Kinder der Elternkombination A₂B × A₅, und von ihnen war A₂B Ausscheider. A₅ ergab keine Hemmung. 2 Geschwister A₂ und B waren auch Ausscheider. Tabelle 6 zeigt die Hemmung für einen A₅B-Ausscheider.

Tabelle 6.
Agglutinationshemmungsversuch mit dem Speichel eines Individuums
des Typus A₅B.

	Iso-Serum	Blk.	Hemmungstiter n.												
			1	2	3	4	5	6	7	8	9	10	11	12	13
Speichelpr.	Anti-A	A ₁	+(+)	++	+++	++++	+++
von ♂ M.	»	A ₂	±	+	+(+)	++	+++	+++
Typus A ₅ B...	Anti-B	B	0	0	0	0	0	0	0	0	±	+	++	+++	+++

Die geringe Antigenmenge im Speichel von A₅-Individuen lässt sich, wie schon unter A₄ angeführt, vielleicht dadurch erklären, dass das A-Gen, das einen schwachen Rezeptor in den Blk. bedingt, auch mit dem komplementären Gen S im Wasser auflösliches Antigen nur im geringen Masse in den Organen und somit in den Se- und Exkreten entwickelt.

Rubrizierung der in den letzteren Jahren beschriebenen Fälle schwacher A-Rezeptoren.

Die in dem früheren Artikel¹⁾ erwähnten schwachen A-Rezeptoren, die in den letzten Jahren beschrieben worden sind, müssen bei Berücksichtigung der oben angeführten serologischen Kennzeichen für A₃, A₄ und A₅ zu den unten angeführten schematischen Typen gerechnet werden.

	Typus	Ist nach Ansicht des Verf. anzusehen als Typus
Fischer und Hahn (1935)	A _x	A ₄
Friedenreich (1936) (Fam. 1, hier J.)	A ₃	A ₄
Gammelgaard und Marcussen (1940)	A ₄	A ₅
Hirschfeld und Amzel (1940)	A ₄	A ₄
O. Hartmann u. m. (1941)	A ₃	A ₅
Morawiecki (1941)	A ₄	A ₄
Kammann (1942)	A ₃ B	A ₄ B
Wiener und Silvermann (1941)	A ₃	A ₃
Dahr (1942)	A ₃	A ₃

Anwendung von O-Seren bei Bestimmung schwacher A-Rezeptoren.

Fischer und Hahn (6) haben hervorgehoben, dass O-Seren mit demselben Anti-A-Titer wie B-Seren schwache A-Rezeptoren mit grösserer Intensität agglutinieren als die B-Seren. Dies habe ich bestätigt gefunden, indem ich festgestellt habe, dass eine Minderzahl von O-Seren A₃-, A₄- und A₅-Blk. stärker agglutiniert als B-Seren mit demselben Titer. Diese O-Seren agglutinieren ferner A₃-Blk. ohne das charakteristische Bild, indem sie eine sehr schwache Agglutination der in B-Seren unagglutinierten Blk. bewirken.

So gelingt es nicht bei Schüttelversuchen mit A₃-Blk. in einem solchen O-Serum, unagglutinierte Blk. auszuschütteln. Werden dagegen A₃-Blk. aus einem B-Serum ausgeschüttelt, zeigt es sich, dass die ausgeschüttelten Blk., die wie früher gezeigt in dem zum Schüttelversuch angewendeten Serum nicht agglutiniert wurden, in einem O-Serum mit der oben erwähnten Eigenschaft und mit demselben Titer wie das beim Schüttelversuch angewendete B-Serum nunmehr agglutiniert werden. Die Agglutination der ausgeschüttelten Blk. im O-Serum ist ohne das charakteristische Bild und schwächer als die Agglutination der »unbehandelten« Blk.

Gewisse O-Seren scheinen somit eine Fraktion von A-Antistoffen zu enthalten, die grössere Avidität besitzen als die in den B-Seren. Andere O-Seren verhalten sich genau so wie B-Seren.

¹⁾ Acta path. et microbiol. Scand. 21, 535, 1944.

Es ist wichtig zur Diagnostizierung des A_3 -Typus, dieses Verhalten zu kennen. Es erklärt, weshalb Dahr (4) bei der Beschreibung eines A_3 -Falles eine Variation in der Agglutinationsintensität findet, die in keinem Verhältnis zur Stärke der angewendeten Seren steht.

Immunisierungsversuche mit A_3 -, A_4 - und A_5 -Blk.

20 Kaninchen mit präformiertem Anti-A im Serum wurden auf die Weise immunisiert, dass 4 von ihnen A_3 -Blk., 8 A_4 -Blk. und 8 A_5 -Blk. erhielten, 1 cm³ 50 % jeden 2. Tag 3 Wochen lang. 2 Wochen später wurden die Kaninchen wieder auf dieselbe Art immunisiert, wobei jedoch die Hälfte der Kaninchen jeder Gruppe mit A_1 -Blk. immunisiert wurde. Sowohl nach der 1. als nach der 2. Immunisierung wurden die Kaninchenserum auf ihren Anti-A-Gehalt untersucht, indem sie gegenüber A_1 -, A_2 -, A_3 -, A_4 - und A_5 -Blk. ausitriert wurden.

Es zeigte sich dabei, dass die hergestellten Seren qualitativ einheitlich waren, indem sie alle denselben quantitativen Unterschied in der Agglutination von Blk. der verschiedenen A-Typen aufwiesen. Eigentümlich war es zu beobachten, dass A_3 -Blk. selbst im Serum, das durch Immunisierung mit diesen hergestellt war, in dem gewöhnlichen charakteristischen Bild und auffällig schwach agglutinierten.

Quantitativ differierte die Menge des Anti-A in den hergestellten Immunseren kaum mehr, als was individuellen Unterschieden bei den Kaninchen entsprechen kann. Kaninchen scheinen somit ein einheitlich zusammengesetztes Anti-A zu bilden, das von dem zur Immunisierung verwendeten A-Typus unabhängig ist.

Der Bluttypus A_x .

Bei den Typenbestimmungen, die wie erwähnt am Staatlichen Seruminstitut vorgenommen wurden, war ein Fall, wo die Blk. weder bei der ersten noch der zweiten Bestimmung mit Iso-B-Seren agglutinierten und wo im Serum nur Anti-B nachgewiesen werden konnte.

Bei Untersuchung in kräftigem Iso-B-Serum und in Anti-A-Immunserum agglutinierten die Blk. schwach. Die Familienuntersuchung ergab keinen Anhaltspunkt für einen durch Erbanlage bedingten Rezeptor, und nach den serologischen Reaktionen scheint es nicht angezeigt, ihn als ein 6. Glied der A-Reihe aufzufassen, weshalb er als ein isolierter Fall zu betrachten ist und im folgenden als A_x bezeichnet wird.

Bei einer Agglutinationsuntersuchung zeigte es sich, dass A_x -Blk. in den besonders agglutininreichen Iso-B-Seren mit fast derselben Intensität agglutinierten wie A_6 B-Blk. Ein direkter Vergleich liegt nicht vor, aber eine Agglutination war nur in Serum J (Titer 1000 bis 2000) und in Serum U (Titer 512) nachzuweisen, und zwar war

die Agglutination im Serum J stärker als im Serum U und entsprach an Stärke der bei A_5B beobachteten Agglutination in denselben Seren. In Anti-A-Immunseren agglutinierten A_x -Blk. ebenfalls und ebenso stark wie A_5B . Dass es sich in den Immunseren nicht um eine Agglutination mit Nicht-Anti-A-Agglutininen gehandelt hat, ist dadurch wahrscheinlich gemacht, dass die Immunseren mit Blk. von 10 Individuen des Typus O keine Reaktion aufwiesen. Verglichen mit A_5 agglutinierten die A_x -Blk. in O-Seren schwächer als jene, aber was die Stärke der Seren betrifft, parallel mit den A_5 -Blk., so dass in O-Seren, wo A_5 kräftiger agglutinierte als in B-Seren, auch die Agglutinationsintensität von A_x im selben Verhältnis stieg. Durch Absorptionsversuche war es nicht möglich, trotz Absorption mit 1 Vol. Blk. einen Titerfall durch Austitrierung gegenüber A_1 - oder A_2 -Blk. festzustellen. Eine geringe Abnahme der Agglutinationsintensität in den zunehmenden Verdünnungen des absorbierten Serums war nicht mehr, als was eine gleichzeitig vorgenommene Absorption mit O-Blk. ergab.

In Anti-O-hältigen Ochsenseren zeigten die A_x -Blk. die Eigentümlichkeit, dass sie in diesen überhaupt nicht agglutinierten und durch Absorption nicht imstande waren, Anti-O zu entfernen. A_x wurde im Laufe eines Jahres zu wiederholten Malen untersucht, und es sind mindestens 10 Anti-O-hältige Ochsenseren, in denen A_x -Blk. nicht agglutinierten, während die Agglutination mit O-Blk. die Stärke ++ bis +++ auf dem Objektglas aufwies. Nach den früher erwähnten theoretischen Erwägungen über die Entwicklung der O-Substanz sollte man bei einem so schwachen A-Rezeptor einen deutlich nachweisbaren O-Rezeptor erwarten.

Eine Schwächung der Agglutinabilität der Blk. im Allgemeinen kann natürlich die sehr schwache Agglutination in Anti-A-Seren und die fehlende Agglutination in den schwächeren Anti-O-Seren erklären, aber nicht die fehlende Absorptionsfähigkeit. Übrigens haben wiederholte Untersuchungen an frisch entnommenen Blutproben immer dasselbe Ergebnis gehabt, und ferner hat eine Agglutinationsuntersuchung in Anti-M- und Anti-N-Seren gezeigt, dass die Blk. vom Typus MN waren und mit derselben Stärke agglutiniert wurden wie Kontroll-MN-Blk.

Bei wiederholten Untersuchungen des Serums von A_x konnte nie Anti-A nachgewiesen werden, nicht einmal bei 4°, während das Serum auf dem Objektglas maximale Agglutination mit B-Blk. ergab.

Ferner ist die hemmende Einwirkung des Speichels auf die Agglutination in Anti-A- und Anti-B-Serum untersucht worden, und es hat sich dabei konstant eine stark hemmende Wirkung auf die Agglutination in B-Seren gezeigt, was aus Tabelle 7 hervorgeht.

Tabelle 7.

Agglutinationshemmungsversuch mit dem Speichel eines Individuums des Typus A_x .

	Iso Serum	Blk.	Hemmungstiter n.											
			1	2	3	4	5	6	7	8	9	10	11	12
Speichelpr.	Anti-A	A_1	0	0	0	0	0	0	0	0	±	+	++	+++
von ♂ H.	Anti-A	A_2	0	0	0	0	0	0	0	0	0	0	±	+
Typus A_x ..	Anti-B	B	++	+++	+++	+++

Der Speichel von A_x zeigt somit einen A-Antigengehalt, der sich mit dem A-Antigengehalt des Speichels von A_1 - und A_2 -Ausscheidern messen kann, und gar nicht den Werten entspricht, die bei schwachen A-Typen wie A_4 und A_5 gefunden worden sind.

Es sind Typenbestimmungen der Familie vorgenommen worden. A_x hat 5 Geschwister, von denen 2 : A_2 und 3 : O sind. Die 2 A_2 zeigten in jeder Beziehung normale Reaktionen, enthielten kein irreguläres a_1 , wiesen mit Anti-O-Serum dieselbe Reaktion auf wie Standard A_2 -Blk. und absorbierten auch wie Standard A_2 -Blk. Die Blk. der 3 O-Individuen ergaben alle mit Anti-O eine kräftigere Agglutination als die 2 A_2 , und im Serum wurde ein kräftiges Anti-A und Anti-B nachgewiesen. Die Blk. zeigten keine Agglutination mit den Seren, in denen A_x agglutinierte. Der Speichel konnte nur von dem einen der 3 O-Individuen untersucht werden: hier zeigte sich keine Hemmung der Agglutination in Anti-A- und Anti-B-Seren.

Die Eltern sind tot. Soll A_x auf einem selbständigen allelomorphen A-Gen beruhen, müssen die Genotypen der Eltern $A_2O \times A_xO$ gewesen sein. Die 2 A_2 -Geschwister von A_x können deshalb genotypisch ganz gut entweder A_2O oder A_2A_x sein, aber leider hatte nur der eine ein Kind, und dessen Typus A_2 gab keinen Aufschluss über den Genotypus des Vaters. Von Verwandten seiner Mutter wurden nur 2 ihrer Geschwister untersucht, sie waren typische A_2 und A_2B . Väterlicherseits, wo der Hypothese zufolge A_x vorkommen sollte, waren alle Geschwister gestorben, und die Untersuchung ihrer Kinder gab keinen Anhaltspunkt für die Existenz eines A_x -Typus in dieser Familie.

Durch die Familienuntersuchung konnte die Möglichkeit eines selbständigen Gens für A_x weder bekräftigt noch widerlegt werden.

Die serologischen Reaktionen lassen es aber nicht angezeigt erscheinen, A_x als ein 6. Glied in die A-Reihe einzugliedern, da die fehlende Anti-O-Reaktion und der kräftigere A-Antigengehalt des Speichels der für alle A_3 — A_5 nachgewiesenen kräftigen Agglutination mit Anti-O-Serum und der für alle 3 Typen beobachteten Abnahme des A-Antigengehaltes des Speichels widerspricht. Ferner ist das Feh-

len eines irregulären Agglutinins im Serum bei einem schwächeren Typus als A_6 nicht zu erwarten, jedenfalls nicht bei tiefen Temperaturen, wenn sich die von A_3 bis A_5 beobachtete Zunahme auch weiter fortsetzen soll. Mit einigem Vorbehalt kann man deshalb vielleicht geltend machen, dass das Fehlen eines irregulären Agglutinins bei x_A gegen die Auffassung desselben als eine 6. allelomorphe A-Eigenschaft spricht.

Es fragt sich deshalb, ob nicht A_x als defekter A-Typus aufzufassen ist, nach den Typen seiner Geschwister vermutlich A_2 , nur dass das Blk.-Antigen und damit wahrscheinlich das ganze im Alkohol auflösliche Antigensystem phänotypisch nicht zur Entwicklung gekommen ist, entweder wegen Wegfalls normal für die Entwicklung des alkoholauflöslichen Antigensystems vorhandener Gene oder wegen Einwirkung eines rezeptormodifizierenden, in casu abschwächenden Gens auf das alkoholauflösliche Antigensystem. Die Familienuntersuchung hat diese letzte Möglichkeit, dass es sich um ein durch Erbanlage bedingtes, rezeptormodifizierendes Gen handelt, weder bekräftigen noch widerlegen können.

Fälle rudimentärer Entwicklung des alkoholauflöslichen Antigensystems müssen wegen der Stabilität im Erbgang der Bluttypen als sehr selten betrachtet werden. Nur ein einziger früher beschriebener Fall, der Haselhorst'sche, Mutter A_2B , Kind O, könnte auf eine mangelhafte Entwicklung des alkoholauflöslichen Antigensystems beim Kind schliessen lassen, und wenn wir A_x in Betracht ziehen, scheint die Annahme natürlich, dass es das A-Antigen ist, das mangelhaft entwickelt ist, und dass es sich um einen Typus A_x handeln sollte. Das Resultat der Typenbestimmung von Kind und Mutter ist von Landsteiner, Schiff und Sachs bestätigt worden. Blutproben des Kindes wurden, bis das Kind 2 Jahre alt war, mehrmals untersucht. Weder durch Absorptions- noch durch Agglutinationsuntersuchungen konnte irgend eine Reaktion in Anti-A- oder Anti-B-Seren nachgewiesen werden. Im Serum des Kindes wurde sowohl Anti-A als Anti-B gefunden, die Stärke ist jedoch nicht angegeben. Eine Agglutinationsuntersuchung in Anti-O-hältigem Serum und ein Versuch, den Antigengehalt des Sekrets zu bestimmen, ist nicht vorgenommen worden. 1934 (das Kind war damals ca. 5 Jahre alt) hat Haselhorst abermals eine Typenbestimmung vorgenommen. Das Resultat ist von Moureau (28) berichtet worden, aber er gibt nur an, dass kein Rezeptor in den Blk. des Kindes nachgewiesen wurde.

Es wäre von Interesse, wenn der Haselhorst'sche Fall nochmals einer Untersuchung unterzogen würde, speziell mit Hinblick auf einen möglichen Antigenhalt des Sekretes und eine Reaktion der Blk. mit Anti-O-hältigem Serum.

Zusammenfassung.

1) Es wurden 10 Individuen der Typus A_4 bestimmt. A_4 ist durch einen quantitativ schwächeren Rezeptor als A_2 gekennzeichnet, ca. 8-mal so schwach, etwas schwächer als der A-Rezeptor bei A_2B .

Im Serum von 10 A_4 -Individuen wird in 4 Fällen irreguläres α_1 bei Zimmertemperatur nachgewiesen. In Anti-O-hältigem Ochsen Serum agglutinieren A_4 -Blk. mit annähernd derselben Stärke wie A_3 .

Familie 1 (hier Familie J.) aus Friedenreichs A_3 -Material ist eine A_4 -Familie.

3 A_4 -Familien machen es wahrscheinlich, dass sich A_4 auf Basis eines allelomorphen A-Gens vererbt.

Im Speichel von 19 A_4 -Individuen wird eine so geringe Menge A-Antigen nachgewiesen, dass sie alle zur Nicht-Ausscheidergruppe zu rechnen sind.

2) Der A_5 -Typus ist der früher von Gammelgaard und Marcussen in der Zeitschr. f. Immunitätsf. 98: 411, 1940 als A_4 beschriebene Rezeptor. Weitere 3 Fälle sind bestimmt worden.

A_5 ist ein noch schwächerer A-Rezeptor als A_4 , ca. 8-mal so schwach.

Was die serologischen Verhältnisse betrifft, sei übrigens auf den früheren Artikel verwiesen.

Im Speichel von 18 A_5 -Individuen wird eine so geringe Menge Antigen nachgewiesen, dass sie alle in die Nicht-Ausscheidergruppe fallen.

3) Gewisse O-Seren verwischen das für A_3 charakteristische Agglutinationsbild, indem sie mit den in B-Seren unagglutinierten Blk. eine schwache Agglutination ergeben. Diese O-Seren agglutinieren die A_4 - und A_5 -Blk. kräftiger als B-Seren mit demselben Titer. Andere O-Seren verhalten sich wie B-Seren.

4) Immunisierung von Kaninchen mit A_3 -, A_4 - und A_5 -Blk. ergibt qualitativ und quantitativ den gleichen A-Antistoff.

5) A_x ist durch einen A-Rezeptorgehalt der Blk. in derselben Höhe wie A_5B gekennzeichnet. Kein α_1 im Serum. Keine Reaktion mit Anti-O. A-Antigen im Speichel mengenmässig ebenso viel wie bei A_1 -Ausscheidern. Es wird die Möglichkeit erörtert, dass es sich um einen defekten A-Typus handelt, bei dem das alkohollösliche Antigen-system in der Entwicklung gehemmt ist, während das wasserlösliche natürlich entwickelt ist, entweder wegen Wegfalls normal für die Entwicklung des alkoholauflösligen Antigensystems vorhandener Gene oder wegen Einwirkung eines rezeptormodifizierenden Gens auf das alkoholauflöslige Antigen-system.

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TRAUMA AND TUBERCULOSIS*)

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(Received for publication June 23rd, 1943).

The question as to whether a traumatic injury may give rise to bone and joint tuberculosis has been discussed since the days of Hippocrates. In the past centuries experienced clinicians have repeatedly called attention to the observation that tuberculosis of joints and bones strikingly often developed in so near relation to traumatic injuries of various nature that a causal connection appeared unquestionable.

Naturally, the discovery of the tubercle bacillus invalidated the theory about the significance of traumatic injury in this respect — at any rate the view that cases of this kind were due entirely to traumatism. Still, the clinical connection between the two conditions seemed yet so striking that the idea about the significance of the trauma was not given up altogether, and clinicians kept looking for an explanation of this effect of traumatic injury. Some assumed that the trauma produced a *locus minoris resistentiae*, more susceptible to hematogenous infection than normal tissue; others advocated the view that traumatic injury activated a preexisting small and symptom-free tuberculous focus — a so-called latent focus — which would not otherwise have developed into a real tuberculous lesion.

Even today, opinions differ as to the significance of traumatic injury to the appearance of tuberculous foci in joints and bones. Some experienced clinicians hold that this significance of trauma is unquestionable — *e. g.*, in the Scandinavian countries, Sinding-Larsen and Johan Holst¹⁾, while others think that this asserted role of the trauma is illusory (*e. g.*, Hans Thomsen²⁾).

To-night it will be my task as an introduction for a discussion to present some considerations concerning this problem. It would be going too far if I here should give a comprehensive review of the con-

*) Read before the Danish Pathological Association on January 20, 1943.

¹⁾ Nordisk Lærebog i Kirurgi. Copenhagen. 1920, 2, 1932.

²⁾ Bibliothek f. Læger. June and July 1942.

flicting arguments and views advanced concerning this problem. I will have to limit myself to mention the facts and considerations which I take to be of essential significance in judging of this question.

To begin with, it will be appropriate to cite a couple of cases of the character which for centuries has made clinicians reckon with the concept »traumatic tuberculosis«.

Case 1.

The patient is a man, 23 years old, who in 1937 was operated on for tuberculous adenitis of the neck. After this, he was perfectly well until June 1939,



Fig. 1.

when he fell down from a scaffold — a fall of 5 meters — landing directly on the buttocks. He was hospitalized at once, and the X-ray examination showed small fractures of two lumbar vertebrae but no sign of spondylitis. (Fig. 1). He was confined to bed for two months. When he got up he still had pain in the back and on this account the vertebral column was roentgenographed again in January 1940, that is, 7 months after the traumatic injury. Now the X-ray picture showed beginning spondylitis of the fractured vertebrae, and he has been under treatment since for this lesion (Fig. 2). In 1941 tubercle bacilli were demonstrated in gastric lavage, but otherwise there were no definite signs of pulmonary tuberculosis. In 1942 a little »cold abscess« was found in the abdomen.

Case 2.

The patient is a woman, 23 years old, with a past history of good health until August 1940 when she fell down five steps of a stairway, which ended

with an outer door, receiving a hard blow under the left breast by the door handle, so that she sat down heavily on the bottom step. After this accident she felt rather poorly, but she was soon able again to go on with her work again. Sugillations developed under the left breast, but they disappeared again completely. She was then feeling well for about 4 months, whereafter she noticed a little lump on exactly the same place where the door handle had hit her chest, and subsequently this lump turned into an abscess that went on to fistulation. Histological examination of granulation tissue removed from this fistula showed the presence of tuberculosis. Two months later, that is, 6 months after the traumatic injury, the patient commenced to have pain in the lower back, but at that time the X-ray examination showed no sign of spondylitis. This pain did not subside. Yet no X-ray examination of the vertebral column was performed again until January 1942 — 1½ years



Fig. 2.

after the traumatic injury, and now the picture showed a typical spondylitis involving the 4' and 5' lumbar vertebrae. She is still under treatment for this lesion, and recently a «cold abscess» has developed in the abdomen.

Case 3.

This patient is a man, 52 years old, whose past history is negative as to tuberculous affections. He had been perfectly well until August 1939 when a heavy board fell from a height of 2 meters, landing perpendicularly on his right wrist. Swelling and tenderness of the wrist appeared at once, but roentgenography of the joint showed no abnormality. The swelling and tenderness did not subside completely, and caused considerable inconvenience in his work. On this account the wrist was roentgenographed again in February 1940, that is, 6 months after the traumatic injury. The X-ray picture

showed tuberculosis of the wrist. Subsequently fistulation developed, and tubercle bacilli were found in the pus. He has since been under treatment for tuberculosis of the wrist.

These cases are by no means particularly selected (except in the sense that tuberculosis in a fracture is a rare phenomenon). In any clinic for surgical tuberculosis, not a few cases of this kind will be seen in the course of time. The present cases were picked out as examples chiefly because in all three cases the region involved was roentgenographed at the time of the injury or shortly after, and in all three cases the early X-ray pictures showed normal conditions. In these cases, then, there was at any rate no tuberculous lesion prior to the traumatic injury. Besides, we have a well-established history — or a convincing description — of a powerful blow; and in all three cases the post-traumatic lesion developed soon or shortly after the traumatic injury and exactly at the site of the blow. Finally, in every case, the lesion is unquestionably tuberculosis.

Naturally, such case histories cannot prove anything concerning a causal connection between the injury and the subsequent tuberculosis. It will always be possible to claim that the tuberculous focus would have developed at the same juncture, even if the accident had not taken place.

The question then arises whether statistical accounts may give any decisive answer to the question.

Can it possibly be shown that the frequency of joint and bone tuberculosis in persons who have suffered traumatic injuries of various nature is greater than the average frequency of such lesions in general?

Hans Thomsen states that the incidence of joint and bone tuberculosis in Denmark amounts to about 150 new cases a year — a number which I find surprisingly low. I have no other figures at my disposal, however, and even if the frequency were 4—5 times greater it would make no difference to the following arguments.

Of these 150 patients, at any rate, not over 20 %, that is, not over 30 patients give a history of traumatic injury; and a really convincing traumatic history will undoubtedly not be present in more than 10 out of the 150 cases. Accordingly, the incidence of joint and bone tuberculosis in this country is only one new case a year per 30,000 inhabitants, and only one clinically convincing instance of traumatic tuberculosis per 400,000 inhabitants.

It is obvious that a statistical investigation into the frequency of such an uncommon lesion in various traumatic materials — *e. g.*, fractures of the spinal column or distortion of the ankle — is not likely to give convincing results. Even if we had at our disposal a material of 5000 cases, each of which had been under observation at least one year, we would have only a small chance of meeting with merely 1 out of the 10 cases of so-called traumatic tuberculosis that

turn up in the course of one year among the 4 million inhabitants of this country. More likely, we would meet with no instance of joint and bone tuberculosis among the 5000 cases of traumatic injury, but this would not justify the conclusion that traumatic tuberculosis is an illusory phenomenon. Even one case of this kind among 10,000 would mean a higher incidence, indeed, than is observed in the entire population. So, I think, we have to realize that it is impracticable to obtain any reliable statistical information about this question when the frequency of the lesion is so slight.

The »opponents« of traumatic tuberculosis often cite a statistical account from a German hospital: Bergmannsheil-Bochum. In this hospital, through a period of 10 years, there was not a single instance of joint and bone tuberculosis in a material of 4000 patients who had suffered some injury by accident. This means that about 400 patients were observed yearly — presumably with an observation period that did not exceed one year. On observation of 400 persons from the Danish population in general per year, in the course of a hundred years we would observe the appearance of one case of joint and bone tuberculosis — and if we were energetic enough to continue this investigation for a thousand years we would be able to observe the appearance of one clinically typical case of traumatic tuberculosis. These figures illustrate properly the small 10-year statistics just mentioned.

Another statistical account reports that during a period of 6 years only 6 cases of joint and bone tuberculosis were notified among 1.6 million members of German sports clubs. Among 1.6 million individuals of the general population, about 300 cases of joint and bone tuberculosis would develop in the course of six years and at any rate 30 of these patients would give a history of traumatic injury. Furthermore, the 1.6 million sportsmen are just in an ageclass where tuberculous lesions develop relatively frequently, so that the small number of notified cases must be due to some defect in the statistics.

As a matter of fact, then, the significance of the traumatic injury cannot be proved casuistically, and statistically it will at any rate be very difficult to obtain any informative figures. As far as I know, hitherto it has not been possible by statistical accounts to settle decisively the question about the significance of traumatic injury to the appearance of joint and bone tuberculosis.

So we still have to reckon with the possibility that traumatic injury may be of significance in this respect.

The question then arises, whether there be no other observations and experiences — besides the casuistic and statistic — that we may take as supporting evidence when we wish to form some opinion about this matter.

Searching for evidence of this kind, it seems obvious to consider our present knowledge concerning the appearance of clinically mani-

fest tuberculosis and the effect of traumatic injury on preexisting tuberculous processes.

We all know that infection with tubercle bacilli in man does not necessarily mean that the clinical tuberculous lesion will develop. Most often, I think, the initial tuberculous infection gives no symptoms — or at any rate, the symptoms are not recognized as tuberculous. Indeed, the morbid processes produced by the initial infection — the primary complex — subside completely even in a great many cases, or they can be demonstrated only with difficulty. In these cases a persistent tuberculin reaction is the only sign of remnants of the infection somewhere in the organism.

According to our present knowledge — based, among others, on the experimental studies reported by Winge¹⁾ — a positive tuberculin reaction means the presence of tubercles somewhere in the organism, and the persistence of a positive tuberculin reaction thus means a persistent presence of tubercles, which — as far as we know — is not possible without living tubercle bacilli.

In many acute non-tuberculous infections the organism either overcomes the infection completely, or the infection conquers eventually the infected organism. But when a person is infected with tubercle bacilli, then, as a rule, neither of the two events takes place: on the contrary a balance is brought about between the infection and the defensive forces of the organism, resulting in the formation of a few tubercles encircling small groups of tubercle bacilli; and these few tubercles may be located here and there in the organism, apparently without affecting the organism, and without the tubercle bacilli being destroyed. This state of balance is extraordinarily characteristic of the tuberculous infection, and it may last for years — nay, often even throughout life.

As the primary complex is localized to the respiratory organs or to the digestive tract, the small latent tubercles will most often be found in these organs, especially in the regional lymph glands of the respective organs, and here they have been demonstrated innumerable times in autopsy on patients who died of some disease other than tuberculosis.

But in some of the infected individuals this state of equilibrium becomes upset, so that the infection again gets the upper hand with the result that the tuberculous lesion develops and manifests itself clinically. The equilibrium may be upset, for instance, when the infection, so to speak, is reinforced by a superinfection. Of course, a spontaneous increase in the virulence of the few tubercle bacilli present in the small foci might also disturb the balance. But, as far as

¹⁾ Undersøgelser over Vævsforandringer og Immunitetsreaktioner fremkaldt af dræbte Tuberkelbaciller. Copenhagen 1934.

I know, no such increased virulence has ever been demonstrated or rendered probable.

On the other hand, of course, the equilibrium may also be upset by impairment of the defensive forces of the organism from some cause or other. If so, either a larger tuberculous focus will develop round the site where the small tubercles have been present but latent for a long time or — and perhaps this happens more frequently — the small latent foci form the starting point for a hematogenous spreading of the infection, resulting in tuberculous processes elsewhere in the organism.

In many cases it is not practicable to decide what it is that makes a hitherto latent tuberculous infection suddenly manifest itself as a tuberculous lesion. In the course of time, however, clinical investigators have called attention to various factors that appear to lower the resistance of the organism to tuberculosis. Hübschmann¹⁾ mentions such factors as: pregnancy, advanced age, puberty, undernourishment — poor social conditions in general — measles, influenza, etc. The significance of these factors as well as that of traumatic injury has been emphasized by many experienced clinicians. But if we try to prove the significance statistically we meet with the same difficulties as when we try by means of statistics to demonstrate the significance of traumatic injury to the appearance of joint and bone tuberculosis.

If, for instance, in a material of tuberculous lesions we find that in a good many cases this lesion commenced in connection with one of the mentioned factors, it may very well be asserted that it is merely an accidental coincidence — for one thing, lesions as influenza and measles are very common phenomena. If, on the other hand, we wish to find out how often tuberculosis develops in connection with other diseases, for instance, measles or influenza, it is to be kept in mind that materials of these infectious diseases have to be extraordinarily large because tuberculosis in comparison to these lesions is relatively rare. For the sake of illustration, I may mention that in this country about 8 new cases of pulmonary tuberculosis develop yearly per 10,000 inhabitants. In an epidemic of influenza the incidence of complicating pulmonary tuberculosis may be very low and still considerably higher than in an ordinary average of the population. In order to be of statistical significance, I think, a material of this kind will have to comprise at least 10,000 cases of measles or influenza — a number that will be difficult to obtain.

I have discussed these aspects of the question at some length because I wish to point out a couple of characteristic features in tuberculosis which presumably are well-known to everybody and yet have

¹⁾ Pathologische Anatomie d. Tuberculose. Berlin 1928.

to be mentioned in this connection, as they are of essential significance to the present subject.

In the first place, a tuberculous lesion in man will practically always make its appearance in an organism that has been infected for some length of time, shorter or longer, with tubercle bacilli, and in which the infection has not given any symptoms and often has been demonstrable only by means of the tuberculin test. In these cases, it may rightly be said that tuberculous infection has been present in a latent form long before the manifestation of the tuberculous affection proper.

In the second place, experienced clinicians have pointed out that many different factors may bring about the change of latent tuberculous infection into manifest clinical tuberculosis. Among such factors, besides traumatic injury, mention has to be made of pregnancy, certain age-classes, acute infectious diseases and undernourishment — but the significance of these factors is still a subject of discussion.

We may then turn to consider the clinically manifest tuberculous lesion.

As long as the lesion is active and progressive it signifies that the equilibrium between the tuberculous infection and the defensive forces of the organism is upset, the infection having gained the upper hand for the time being. But, what measures do the clinicians take in order to reestablish the lost balance so that the tuberculous process again becomes quiescent, leaving merely an inactive tuberculous focus together with the irreparable changes brought about by the active lesion?

The clinicians try to strengthen the defensive forces of the organism — and they try to attain this just by counteracting the very factors which a clinical judgment indicates as contributory to the appearance of the tuberculous lesion. They counteract the undernourishment and a poor general condition of the patient by submitting him to the so-called conservative general treatment — most often in the form of sanatorium treatment with plenty of good food, rest in the open, and sun and light treatment. If pregnancy is present it is interrupted; and the clinicians try to protect the patient from intercurrent infection.

Last, but not least, the clinicians try to immobilize that part of the body in which the tuberculous focus is located. Above all, the patient is put to bed, which probably is our most effective form for immobilization. Then attempts are made to obtain more local immobilization — in pulmonary tuberculosis by means of artificial pneumothorax or some other collapse therapy (which serves other purposes too), in joint and bone tuberculosis by means of splints, bandages, plaster casts, etc. Of course, the purpose of the immobilization is to secure a period of rest for the tuberculous process and for the organ in which it is located, protect it against movements and strain — in

brief, against traumata of any kind. I wish to point out that this form of treatment is employed not only in cases of tuberculous inflammation, and that it probably is the most important measure in the conservative treatment of every kind of inflammation. It will suffice here to remind of the conservative treatment of furunculosis and panaritium or the opium treatment of acute appendicitis.

I hardly think that anybody would claim that the outlined treatment of tuberculous inflammation — and this applies in part to inflammation in general — is ineffective. I do not think that any circumstantial documentation of this will be required for the physician who has had occasion himself to observe its effect — and in particular, a chance to observe how the neglect of these principles of treatment at once brings its own punishment. I do not think that any physician nowadays could imagine to neglect the general treatment of a patient with a so-called local tuberculosis. The risk of doing this is well known. If, for instance, in a case of joint and bone tuberculosis the physician neglects immobilization and allows the affected part of the body to be exposed to excessive motion, hard strain or real traumatic injury, his experiences will be sad, as the result will be an aggravation of the condition or an unquestionable relapse.

Here I think it will be appropriate to cite a couple of cases in which a traumatic injury caused a relapse of a joint and bone tuberculosis which clinically and roentgenographically appeared to be quiescent.

Case 4.

The patient is a man who, in 1936, at the age of 18 years, was admitted to the Refsnæs Seashore Hospital for treatment for tuberculous coxitis on the right side. Under conservative treatment the lesion became completely quiescent. The patient got up and walked about for 10 months, while the coxitis roentgenographically kept perfectly unchanged. One day, while walking in the garden of the hospital, the patient stumbled and fell, hitting the ground with the affected hip. This gave him intense pain. Afterwards the pain did not subside completely, and the dynamic power of the leg decreased. On X-ray examination, 1 month after the fall, it could not be decided with certainty whether the roentgenographic features of the coxitis had undergone any changes, but subsequently it was clinically evident that a relapse of the lesion had taken place. A few months later, indeed, the X-ray examination revealed some large, new destructive processes corresponding to the coxitis. On protracted conservative treatment, the lesion became quiescent again so that the patient could be discharged from the hospital in 1942.

Case 5.

In 1937, the patient — a man, 18 years old, commenced to limp a little and have a little pain occasionally in the left hip. In spite of examination in the Orthopedic Hospital and in the Finsen Institute, Copenhagen, no diagnosis could be made with certainty. The X-ray picture showed merely some very slight changes corresponding to the left hip-joint — changes that could not be interpreted with certainty. With our present knowledge of the subsequent course of this affection, however, it is safe to say it was a begin-

ning tuberculous coxitis, even though the X-ray picture kept quite unchanged for about two years.

In the beginning of February 1940 the patient fell off his bicycle and hit the left trochanter hard against the pavement. About a week later his earlier mild hip symptoms commenced to increase rapidly in intensity, and clinical signs of severe coxitis developed within a very short time. Even as early as two weeks after the accident the X-ray picture showed an unquestionable tuberculous coxitis, and only 3 weeks later it showed some large destructive processes in the left hip-joint. Subsequently an abscess appeared on the anterior aspect of the hip, and the presence of tubercle bacilli was demonstrated in the pus from this abscess.

I have picked out these two cases as examples because here a thorough clinical and roentgenological examination has established that the tuberculous processes were present long before the traumatic injury and had kept perfectly quiescent up to the time of the accident. In particular, the X-ray pictures showed no changes whatever in the affection for more than one year prior to the accident.

In both cases the symptoms of the relapse developed immediately after the traumatic injury and were subsequently followed closely by clinical and roentgenological examinations, which showed that in both cases we were dealing with a true relapse associated with an extensive destruction of bone.

Experiences which I think are indisputable show then that improvement of the general condition of the patient and protection from pregnancy and traumatic injury in the widest sense of the term have a favorable influence on any tuberculous lesion present. This treatment contributes to reestablish the equilibrium between infection and defensive forces, presumably by strengthening the latter.

Conversely, it is an indisputable experience that a poor general condition, inadequate immobilization — that is, traumata in the widest sense of the term — and pregnancy have the opposite effect: the lesion is aggravated, and the disproportion between the intensity of the infection and the capacity of the defensive forces is increased.

Now, there is no reason whatever to think that the effect of the factors here mentioned is limited to the clinically manifest tuberculosis. Most likely the poor general condition, pregnancy and traumatic injury have exactly the same affect on the latent symptom-free tuberculous infection, which usually is present many years before the appearance of the tuberculous lesion proper. At any rate, this applies to the more universal factors as the general condition of the individual, pregnancy and infectious disease. As to the effect of traumata, particular conditions assert themselves because the effect of the trauma is local. If the site of the traumatic injury is not also the site of a small tuberculous focus, of course it is not to be expected that the trauma will have any such effect as under discussion here.

For, in this connection, I have not taken the possible significance of a locus minoris resistentiae into consideration at all, as this is a

question of which we know all too little to make its discussion profitable.

Disregarding this possibility, then, the answer to the question whether a traumatic injury to an apparently normal part of the body may produce a tuberculous lesion, will ultimately depend on whether the small latent tubercles, which are present in the organism for years without ever developing into tuberculosis proper, may localize also in the organs where the so-called traumatic tuberculosis arises — for instance, in the joint and bone system.

We therefore have to consider the question whether there is anything to suggest that small latent foci may localize not only in the respiratory organs and the digestive tract, but also in the other organs of the greater circulation.

When this question is raised we will think at once of the theory advanced by Hübschmann¹⁾ about the pathogenesis of the universal miliary tuberculosis.

Hübschmann called attention to the fact that miliary tuberculosis generally excludes the presence of large chronic tuberculous foci. In cases of true general miliary tuberculosis, it was as a rule impossible to demonstrate the starting point for the innumerable tubercle bacilli that produce the miliary tuberculosis of all the various organs. In most cases, autopsy revealed a very small primary complex, rather poor in tubercle bacilli in the lungs and no other source of the many tubercle bacilli scattered throughout the organism. The often mentioned large vascular tubercles in the pulmonary vein were built in such a way that the tubercle bacilli could not originate from them.

An adequate explanation of the presence of the numerous tubercle bacilli distributed in the various organs seemed possible only by assuming that these tubercle bacilli were deposited gradually through several small symptom-free bacteriemias from the small primary focus and then had remained in a latent state till one of the aforementioned factors that weaken the defensive forces of the organism exerted its effect and induced the miliary tuberculosis.

In order to explain the origin of the miliary tuberculosis, then, Hübschmann found it necessary to reckon with the possibility that the tubercle bacilli themselves might be present in a latent state in the organs for some length of time — and as far as I know, no other theory has yet been advanced that offers a better explanation of the particular features of miliary tuberculosis.

Turning from the miliary tuberculosis to the more chronic form of tuberculosis of various organs, we meet also here with some peculiarities that are of interest in this connection.

A thorough review of fairly large materials of joint and bone

¹⁾ Pathologische Anatomie der Tuberculose. Berlin 1925.

tuberculosis, lupus, urogenital tuberculosis, etc., shows¹⁾ that tuberculosis is inclined to a multiple localization in one of the organ systems of the large circulation: either the bone and joint system or the skin or the urogenital system, and leave the others free.

But, when in a patient we find, for instance, 4 joint and bone foci, it is absolutely certain that the other organ systems have been exposed to just as dense a spreading of tubercle bacilli as the joint and bone system; and when both suprarenals show a dense distribution of tubercle bacilli, the other organs supplied by the large circulation must have been subject even to a very dense hematogenous distribution of tubercle bacilli. The question then arises:

What has become of the tubercle bacilli that were deposited in the other organ systems?

In some cases, tuberculous foci make their appearance also outside the organ system which shows a multiple invasion; and in these cases then, the infection overcomes the relative immunity outside the organ system most severely attacked in such a degree that a large tuberculous focus develops.

But what about the other cases in which this does not happen?

Well, for one thing, it is not likely that the defensive forces of the organism in these cases go to the other extreme and immediately kill all the tubercle bacilli that are lodged outside the organ system attacked.

A certain intermediate state seems more probable, namely: the tubercle bacilli are located for some length of time in the organs and — as here we are dealing with an allergic organism — they are walled in by small tubercles which then disappear again after some length of time. In other words: in these cases it is more likely that a number of organs for some length of time contain small latent tuberculous foci.

It is an established fact, then, that tuberculosis often is localized as a multiple process in a single organ system. This justifies the conclusion that in these cases the other organ systems too have been exposed to a hematogenous distribution of tubercle bacilli without presenting any demonstrable tuberculous foci. And the most probable explanation of this will be that small latent foci were formed in these organs and that these foci disappeared again after some length of time. But even though this explanation be the most probable, we cannot be quite sure of its correctness till it has been confirmed by observation.

But, have such small latent tuberculous foci in the organs of the large circulation never been demonstrated by direct observation?

As a matter of fact, the observation of small latent foci in bones

¹⁾ Johs. Meyer: Tuberculose als Organssystemerkrankung. Acta tuberc. Scand. 1935.

and joint capsules will be entirely accidental and occur but seldom, as it will require operation on these organs, which naturally cannot be performed in the absence of symptoms. But, in the case of another organ system — the urogenital system — recently some observations have been reported that have not hitherto been explained adequately, and which probably are to be interpreted in the light of the considerations here presented about the probability of the view that, under certain circumstances, small latent tuberculous foci are bound to occur here and there in the organism.

In September 1939, an international congress of urologists was to have met in New York, and one of its subjects for discussion was »The Early Diagnosis and Treatment of Renal Tuberculosis«. The main papers on this subject were to be read by Wildbolz, of Switzerland, and by Thomas, U. S. A. As far as I know, the congress did not meet but the introductory papers by these two urologists have been published,¹⁾ and they are of very great interest in connection with our subject to-night. After a review of the very extensive literature and an analysis of their own observations, both investigators arrived at the same result, independently of each other.

It is an unquestionable fact that patients suffering from tuberculosis of some other organs — for instance, the lungs or joints or bones — occasionally excrete tubercle bacilli with the urine, and that this tuberculous bacilluria does not signify tuberculosis of the kidney in the classical sense of the term, *i. e.*, a continuously progressing destructive tuberculosis of the kidney, commencing most often in the papillae — a lesion that requires nephrectomy as soon as it is diagnosed if it is unilateral. The first-mentioned bacilluria is not accompanied by pyuria proper, albuminuria or functional impairment of the kidney. Further, it does not give rise to any complaint and hence it is discovered most often merely by chance.

According to Wildbolz, this form of bacilluria is an expression for renal tuberculosis which differs pathologic-anatomically, as well as clinically from the classical tuberculosis of the kidney. On the basis of his studies on kidneys removed operatively and numerous autopsy findings on patients who died of tuberculosis outside the kidney, Wildbolz thinks that the renal tuberculosis in these cases is not localized to the renal medulla but preferably to the cortex. Here in the cortex there are small tubercles which have no tendency to caseation but undergo fibrosis; and Wildbolz designates this condition as »the fibrous form of renal tuberculosis«. Clinically its chief characteristic is a pronounced tendency to recovery, notwithstanding its protracted course. In these cases, then, nephrectomy ought not to be performed even when the bacilluria is unilateral, but the patient is to be treated conservatively under continuous urological observation.

So, this relatively infrequent lesion has been described by American

¹⁾ VII Congress of the International Society of Urology, New York 1939.

as well as European authors, and apparently its presence has been ascertained most often in patients suffering from fairly extensive tuberculosis of organs other than the kidneys.

I think it is justified to say that in patients with this slight and curable form of renal tuberculosis the kidneys are the site of latent tuberculous foci. The pathological changes in the kidney gave no complaints whatever, and they have been demonstrated only through particular search for them. Ordinary clinical examination, especially of the urine, will not reveal any kidney lesion, and cases of this kind are disclosed only by a particular technique of examination. As a matter of fact, it is the more recent advances in the demonstration of tubercle bacilli that have disclosed these cases — for it may hardly be warrantable yet to say »this nosographic unity«.

In this country, too — thanks to Professor K. A. Jensen — we have benefitted by these diagnostic advances, and it is not to be wondered, therefore, that we have had occasion to gain corresponding experiences in the Finsen Institute, Copenhagen, and in the Refsnæs Seashore Hospital. Time does not allow me here to enter into the details of these studies, and it will have to suffice for me to say that the conclusions we now draw from the finding of tubercle bacilli in the urine of our patients — especially patients suffering from joint and bone tuberculosis — differ essentially from those of fifteen years ago. We have seen cases in which an unquestionable excretion of tubercle bacilli with the urine has disappeared again; and we have seen other cases in which autopsy after excretion of tubercle bacilli from the kidneys through a number of years revealed but very small tuberculous foci in the kidneys or none at all — even though the latter finding undoubtedly was due to insufficient examination. Finally, we have not a few patients walking about with periodical or continual excretion of tubercle bacilli but without any other sign of tuberculosis of the kidney — patients in whom nephrectomy will be indicated only if unquestionable signs of a destructive tuberculosis of the kidney should develop subsequently. A few cases of this kind have been described in the dissertation by Tage Kjær on tuberculous bacilluria.¹⁾

In brief: in the recent, very effective, methods for demonstration of tubercle bacilli we now have the means of demonstrating the presence of small latent tuberculous foci in the kidneys — foci that could not be demonstrated before, even though their presence was suspected for more theoretical reasons — while we yet are wanting the means for demonstration of such foci in other organ systems.

Returning to our starting point, the question about the significance of traumatic injury to the occurrence of joint and bone tuberculosis, it will be appropriate, I think, briefly to recapitulate the trend of thought in what I have said:

¹⁾ T. Kjær: Tubercelbaciluri, Kobenhavn 1936.

As a rule, a latent tuberculous infection is present in the organism for some length of time before the tuberculous lesion proper manifests itself clinically. This, I think, can be looked upon as established.

In the course of time, experienced clinicians have called attention to their observation that certain factors — for instance a poor general condition, pregnancy, and traumatic injury — appear to be able to bring about that a latent tuberculous infection develops into clinical manifest tuberculosis. But this has not been proved conclusively.

On the other hand, the therapeutic results obtained in clinical tuberculosis show with certainty that at any rate some of these factors — in particular undernourishment and traumata — have a quite considerable effect on the fully developed clinical tuberculosis: their presence aggravates the state of tuberculosis, and their absence has a favorable effect on it. In particular, traumatic injury in the widest sense of the term — small movements and injury from strain as well as more extensive and gross traumata — have an aggravating effect on tuberculosis as well as on any other inflammatory condition, and the protection against traumata — namely, immobilization — has a curative effect on tuberculosis as well as on other inflammatory conditions.

Now there is no reason whatever why these various factors should not act on the latent tuberculous infection in the same way as on the clinically manifest tuberculosis and thus be able to activate the latent infection into a more extensive tuberculous focus. But if a traumatic injury is to have this effect it naturally will have to strike a tuberculous focus. In particular, if the injury in this way is to produce a clinical joint and bone tuberculosis, the prerequisite will be the location of small latent foci in the joint and bone system. As yet we are not able to prove that this condition is met not infrequently, but several observations indicate that small latent foci now and then must be present for some length of time in the organs of the large circulation, including the joint and bone system. Among these observations I wish to point out especially: 1) the particular conditions met with in the appearance of miliary tuberculosis; 2) the peculiar localization of multiple tuberculosis; and 3) the relative frequent occurrence of symptom-free tuberculous bacilluria. This being the case, it would be most remarkable if a hard blow or some other trauma now and then did not strike such small foci and activate them into a clinical tuberculous lesion, just as well as such a blow may activate a more extensive tuberculous process and make it spread into the surroundings.

So, very diverse observations on the pathological and clinical aspects of tuberculosis have led to the conclusion that under certain circumstances, a traumatic injury is probably able to bring about the appearance of a clinical tuberculous affection. We may even go

so far as to say that if we had not beforehand heard anything about the concept »traumatic tuberculosis« with our present knowledge of the characteristics of tuberculosis we might reasonably expect something of the kind to happen now and then. As traumatic tuberculosis, moreover, has been observed by experienced clinicians through centuries, I think it is fairly safe on the basis of the findings and considerations here advanced to reckon with traumatic tuberculosis as a reality — not a mere illusion.

In conclusion, I merely want to mention that as an argument in favor of their view, the authors who look upon »traumatic tuberculosis« as a mere illusion have pointed out that in cases of this kind the tuberculous process is always localized to the same regions as spontaneous tuberculosis. If, for instance, the traumatic injury hits a knee-joint, the result will never be a skin tuberculosis in the knee regions but always a tuberculous process in the knee-joint itself — in keeping with the fact that tuberculosis of the skin of the extremities is a very rare lesion, whereas tuberculosis of the knee-joint is relatively frequent.

The explanation of this phenomenon, I think, will be that no trauma is able to activate a small tubercle in a tissue which beforehand processes a high natural resistance to tuberculosis. In the striated musculature, for instance, a hematogenous spreading of tubercle bacilli will under no condition give rise to a fairly large tuberculous focus — no matter what agencies the tissue is exposed to. For that matter, I think, small latent tubercles will also be very rare in such tissue.

The traumatic injury thus is able only to activate a tuberculous process in a tissue which beforehand has a certain disposition for tuberculosis. In some cases, presumably, the trauma plays an essential role in the appearance of a tuberculous focus in such a tissue — in other cases it probably acts merely as the drop that makes the cup overflow. So, the coincidence of trauma and tubercle is by no means sufficient for the production of a tuberculous focus. Other conditions — which might be designated as favorable local immunobiological conditions — have to be present if the trauma is to exert its effect in this respect.

This fact has to be realized if we are soberly to estimate the significance of the traumatic injury. But this realization should not conceal the main point in this matter: that reliable observations of widely differing character concerning the clinical and pathological aspects of tuberculosis make it most likely that traumatic injury really may play an essential part in the production of the tuberculous lesion.

INVESTIGATIONS ON THE DURAN-REYNALS SPREADING FACTOR IN STAPHYLOCOCCI

By *Johs. Bøe*, M. D.

(Received for publication 1th Dec. 1943).

In 1928 *Duran-Reynals* (1) found that extracts of various tissues, but particularly extracts of testis, caused a marked increase in tissue permeability. When such an extract was injected into the skin together with a vaccine virus there was a spreading of the local reaction and the general reaction was more severe than without the addition of the extract. The active principle which causes this spreading of the local reaction has since been designated as Duran-Reynals spreading factor and has been the object of detailed investigations.

In 1933 *Duran-Reynals* (2) showed that staphylococci contained a similar spreading factor and that there was a clear relation between the invasiveness of the staphylococci and their content of this spreading factor. Duran-Reynals let the question of the relation of the spreading factor to the other active components of the staphylococci stand open. But since this spreading factor has proved to have a markedly intensifying effect on both staphylococcal infections and other infections, it is reasonable that this has suggested a factor of significance in the pathogenesis of staphylococcal diseases.

The mode of action of staphylococci in the organism is from many aspects not clear, but is generally considered to be determined by the formation of toxins (hemolysin, leucocidin, dermonecrotic toxin, lethal toxin, enterotoxin, fibrinolysin, coagulase, anticoagulant and finally Duran-Reynals spreading factor). But others, for example *Petterson* (3), claim that still other substances (»negataktic substance») determine the pathogenic effect to a great extent. And *Forsmann* (4) and *Flaum* (5) believe that resistance to staphylococcal infections is not determined by antitoxic immunity.

In connection with some work with a large number of pathogenic

and apathogenic staphylococcus strains we investigated the content of Duran-Reynals spreading factor (D-R) in a number of them and it is these observations which are described below.

Technique.

The staphylococcus strains employed were freshly isolated from pathologic processes and the apathogenic ones from normal skin or mucous membrane.

To produce the D-R containing filtrate the strains were inoculated either on agar slants or in broth and incubated for 24 hours. Then the agar slant culture was rinsed in saline solution and filtered through a Zeitz filter. The broth cultures were also filtered in the same manner. The filtrates were employed while fresh.

The rabbits used were albinos which weighed about 2000 gr. They were depilated over a large area on the back and sides with barium sulphide with great caution (Claus Jensens method) and the injections were made intracutaneously on both sides of the back close to the columna. If the reactions were placed farther to the side the spreading was uneven, as it took place for the most part downward towards the sides.

In order to facilitate the demonstration of the spreading, various methods have been used. Thus it is possible to measure the length of time necessary before the intracutaneous blister disappears. The extent of the spreading can be made clearer by adding diphtheria toxin to the filtrate and then measuring the extent of the local reaction. But as a rule dyes have been employed and their spreading in the skin measured. We tried the various methods and of stains we tested gentian violet, kohlschwarz, trypan blue and India Ink. Trypan blue proved to be the most suitable (*von Behring* (6)) and we used it in a 0.75 % solution.

The filtrate to be examined was, in various dilutions, added to equal parts of the stain solution. In some experiments immune serum was also added to the mixture (cf. experiments). The injected fluid quantity was usually 0.10 cc, in some cases 0.20 cc. We found it impractical to use larger doses. Intracutaneous injections of such large doses as 0.75 cc, which some earlier investigators have employed, give less exact results.

The injection was made exactly intracutaneously with a very fine needle. The immediate increase of the blister was observed and the extent of the reaction was usually measured after 45 minutes and 24 hours. Measurement after 45 minutes gives a measure of the speed with which the spreading takes place, while measurement after 24 hours shows the maximum extent of the spreading. Saline solution with the same quantity of trypan blue was used as control, and in some cases an extract of rabbit testis was used as »positive control«.

The largest and smallest diameters of the reactions were measured and the surface of the spreading of the stain was calculated. This accuracy must be taken with certain reservations, but the values express the differences in the size of the reactions which can be readily observed during the experiments.

There is a considerable difference in permeability in the various rabbits, which will appear from the tables. This difference is so great that the reactions cannot be compared from one rabbit to another, while the size of the reactions in any individual rabbit seems to be rather constant.

We therefore found it necessary to carry out each experiment with the required controls on the same rabbit. *Claude and Duran-Reynals* (11) tried to obtain more comparable results by calculating the extent of the reactions in relation to the control reaction for each rabbit (spread of filtrate/spreading of saline), but we were not convinced that we by this method could compare the results from one rabbit to another with any degree of accuracy, and therefore the results are presented for each individual rabbit.

When a number of yellow hemolytic staphylococci strains and, for comparison, some white apathogenic strains were investigated for their capacity to produce D-R, it was found (Table 1) that there was a considerable difference between the individual strains in this respect. And it was not possible to find any correlation between this capacity and the other properties of the strains, or any correlation with their origin. There was furthermore no correlation between their hemolysin production and their capacity to produce D-R. The »Wood« strain which is used in so many laboratories for toxin production was also used for comparison. As the table shows it had a high D-R content but lower than strains 60 and 69 which were much weaker hemolysin producers than the »Wood« strain. It also appears that some yellow pathogenic strains may lack D-R such as our strain 122 which was isolated from a case of rhinitis examined during an aerogenous epidemic of wound infections. This strain was a strong hemolysin producer, coagulated plasma in one hour and fermented mannite in 24 hours.

The white, apathogenic staphylococcus contains insignificant D-R, much less than the yellow hemolytic staphylococci (see also Table 6).

It thus appears that a good toxin producer also produces considerable D-R but there is not necessarily any correlation between these two properties. *McLean* (7) demonstrated analogous relations for anaerobic bacteria. And it appears already from these experiments that there cannot be any identity between hemolysin and D-R.

When, as in the present experiments, testis extract is used as control, there appears to be a certain qualitative difference between the reactions produced with testis extract and filtrate of staphylococcus cultures. The spreading on injection of testis extract is even and the intensity of the stain reaction recedes gradually and has poorly defined limits against the surrounding skin. The spreading on the

Table 4.

The Duran-Reynals spreading factor in filtrates of various strains of staphylococci.

Rab-bit	Intracutaneous injection of filtrate	Spreading	Remarks
B. 9	From str. no 60 (Yellow hemolytic staph. from rhinitis)	820 mm ²	Necrosis in 3 days. Necrosis i 24 hours.
	„ „ „ 69 (Yellow hemolytic staph. from rhinitis)	960 „	
	„ „ „ »Wood« (Old laboratory strain, atoxic filtrate)	720 „	
	„ „ „ »Wood« (Old laboratory. toxic filtrate. LH : 0,10)	750 „	
	Extract of rabbit testis	720 „	
	Saline control	180 „	
B. 10	From str. no 25 (Yellow hemolytic staph. from abscess)	290 mm ²	
	„ „ „ 27 (White apathogenic staph. isolated from air)	180 „	
	Extract of rabbit testis	600 „	
B. 11	From str. no »Wood« (Toxic filtrate. LH : 0,10)	570 mm ²	Necrosis i 3 days.
	„ „ „ 25 (Yellow hemolytic staph. from abscess)	180 „	
	„ „ „ (White apathogenic staph. isolated from air)	130 „	
	Extract of rabbit testis	490 „	
	Autolysate from strain »Wood«	570 „	
	Filtrate from salmonella typhi	80 „	
B. 12	From str. no 84 (Yellow hemolytic staph. from rhinitis)	570 mm ²	
	„ „ „ 85 (Yellow hemolytic staph. from sore throat)	490 „	
	„ „ „ 71 (Yellow hemolytic staph. from sore throat)	430 „	
	„ „ „ 122 (Yellow hemolytic staph. from rhinitis)	310 „	
	Extract of rabbit testis	960 „	
	Saline control	310 „	

Table 1. (cont.)

Rab-bit	Intracutaneous injection of filtrate	Spreading	Remarks
B. 58	From str. no 514 (Yellow hemolytic staph. from abscess)	410 mm ²	
	• • • 504 (Yellow hemolytic staph. from pyodermia)	450 •	
	• • • 25 (Yellow hemolytic staph. from abscess)	310 •	
	• • • 515 (Yellow hemolytic staph. from abscess)	570 •	
	Extract of rabbit testis	710 •	
	Saline control	150 •	

Equal parts of filtrate and a 0,75 % suspension of Trypan blue were injected. The total dose was 0,10 cc. The reactions read after 24 hours.

injection of culture filtrates is more sharply defined against the surrounding skin and the entire area seems more inflamed (toxin reaction) and at the outer limits there is an indication of slight elevation.

Madinaveita (8) believed that the D-R factor in testis extract and in bacteria (perfringens toxin) was identical while *von Behring* (6) regarded this as improbable. According to our experiments we have reason to believe that these are two distinct qualities. In this connection it may be mentioned that *Duran-Reynals* (9) and *McLean* (7)

Table 2.

Influence of antitoxic serum upon the spreading factor in staphylococci.

Rab-bit	Filtrate	Amount of filtrate	Spreading	
			Filtrate + normal rabbit serum 0,10 cc.	Filtrate + antitoxic rabbit serum 0,10 (2 A. E.)
B. 14.	Strain • Wood•	0,03 cc	310 mm ² necrosis	570 mm ² no necrosis
		0,003 •	300 •	570 •
		0,0003 •	250 •	530 •
B. 15	Strain • Wood•	0,03 cc	530 mm ² necrosis	1260 mm ² no necrosis
		0,003 •	530 •	1260 •
		0,0003 •	310 •	510 •
B. 57.	Strain no 514 (from abscess)	0,03 cc	490 mm ²	490 mm ²
		0,003 •	230 •	280 •
		0,0003 •	250 •	250 •

Equal parts of filtrate, serum and Trypan blue were injected intracutaneously. The reactions read after 24 hours.

found that the spreading factor in testis extract had no antigenic properties while *von Behring* (6) and *McLean* (7) who found the same, have demonstrated that the D-R factor in *Cl. perfringens* toxin gives strong antibody production on immunisation. The same is true of D-R in snake venom according to *Duran-Reynals* (10). And *von Behring* who examined this antibody production for a number of anaerobic bacteria found that the neutralising effect of immune sera was specific.

Table 3.

Influence of antibacterial serum upon the spreading factor in staphylococci.

Rab- bit	Filtrate	Amount of filtrate	Spreading			
			Filtrate + normal rabbit serum 0,10		Filtrate + antibacterial rabbit serum 0,10 (agglutinin titre 12800)	
			45 min.	24 hours	45 min.	24 hours
B. 47	Strain no. 298 (from stomatitis)	0,03 cc	620 mm ²	700 mm ²	180 mm ²	700 mm ²
		0,003 "	620 "	700 "	200 "	700 "
		0,0003 "	410 "	700 "	90 "	700 "
B. 48	Strain no. 298 (from stomatitis)	0,03 cc	280 mm ²	280 mm ²	35 mm ²	230 mm ²
		0,003 "	200 "	200 "	45 "	140 "
		0,0003 "	200 "	200 "	35 "	140 "

For explanation see table 2.

The reactions read after 45 min. and 24 hours.

We therefore examined whether the D-R factor in staphylococci could also be neutralised by antitoxic serum.

The table shows that this is not the case. The spreading was not inhibited by the antitoxic serum. However these experiments show a pronounced difference between the dermonecrotic toxin and D-R. While the dermonecrotic toxin which caused necrosis in the experiments with normal rabbit serum were completely neutralised by the antitoxic serum, this serum had no effect on D-R. The difference which the D-R factor in staphylococci here shows from the D-R factor in other microbes may perhaps tell us something of the mode of action of staphylococci in the organism.

It was then examined to what extent a strong antibacterial serum had any effect on the D-R factor in staphylococci. The serum here employed was produced by intravenous immunization with formalin-killed staphylococci over a long period. The agglutinin titre of the serum was 12800.

It appears from the table that this serum had a marked inhibition on the spreading in contrast to the antitoxic serum. This effect is

apparent as soon as the injection has been made and is here measured after 45 minutes when the difference is very pronounced. After 24 hours the difference in spreading is obliterated.

Here it is observed that there is very little difference in the spreading for the various dilutions. This is a constant observation which has also been made by other investigators (cf. *Duran-Reynals* (2)).

Table 4.

Spreading following injection of homologous and heterologous filtrate in a rabbit immunized with formalin killed staphylococci.

Amount of filtrate	Spreading	
	Filtrate from homologous strain (no. 298)	Filtrate from heterologous strain (no. 25)
0,03	540 mm ²	430 mm ²
0,003	300 ,	230 ,
0,0003	120 ,	120 ,

For explanation see table 2.

The reactions read after 24 hours.

We also tried to demonstrate the inhibiting effect on the spreading which the above experiment illustrates by measuring the spreading on the immunised rabbit. We therefore injected filtrates of homologous and heterologous cultures into a rabbit which had an agglutinin titre of 12800.

There was here a pronounced impression that the spreading took place more slowly than in a normal rabbit but this evaluation is neces-

Table 5.

Influence of serum from highly sensitized rabbit upon the spreading factor of staphylococci.

Rabbit	Filtrate	Amount of filtrate	Spreading			
			Filtrate + normal rabbit serum		Filtrate + serum from sensitized Rabbit	
			45 min.	24 hours	45 min.	24 hours
B. 59	Strain no. 315 (from abscess)	0,06	310 mm ²	750 mm ²	250 mm ²	850 mm ²
		0,006	250 ,	710 ,	250 ,	850 ,
		0,0006	200 ,	410 ,	180 ,	410 ,
B. 60	Strain no. 315 (from abscess)	0,06	620 mm ²	850 mm ²	570 mm ²	960 mm ²
		0,006	530 ,	570 ,	490 ,	660 ,
		0,0006	180 ,	490 ,	200 ,	540 ,

For explanation see table 2.

sarily subjective. It is, as mentioned above, inconsequent in these experiments to use one rabbit as control for another because the individual variation is so great. However it seemed that the spreading was retarded just as much when a heterologous serum was employed as a homologous one.

Table 6.

Spreading following injection of staphylococcal filtrates in a rabbit highly sensitized against staphylococci.

Rabbit	Intracutaneous injection	Spreading
B. 50 Reacting titre 1/10000	Filtrate from strain 514 (Yellow hemolytic staph. from abscess)	590 mm ²
	„ „ „ 504 (Yellow hemolytic staph. from pyodermia)	700 „
	„ „ „ 25 (Yellow hemolytic staph. from abscess)	315 „
	„ „ „ 515 (Yellow hemolytic staph. from abscess)	500 „
	„ „ „ 486 (White apathogenic staph. isolated from air)	200 „
	Extract of rabbit testicle	710 „

For explanation see table 1.

Analogous to the experiments shown in Tables 2 and 3 we investigated to what extent antibodies against the spreading factor were present in a highly allergic rabbit. The serum employed was tapped from a rabbit which was sensitized by intracutaneous injection of formalin-killed staphylococci and whose skin titre was 1/10000.

It was not possible to demonstrate any neutralising effect of this serum, there was no delay in spreading. And the maximum spreading was just as large with the addition of normal serum or serum from the allergic rabbit. Nor was there any observable retarding effect when heterologous and homologous filtrates were injected into the highly allergic rabbit.

This last result is rather surprising in view of the considerable capacity of the allergic organism to localize a reaction produced with a formed antigen, for example a suspension of the homologous bacteria.

Summary.

1. A number of yellow hemolytic staphylococcus strains are examined for their content of Duran-Reynals spreading factor. There

seems to be no correlation between the virulence of the strains and their content of this factor. As a rule a filtrate with a high hemolysin content also has a high content of the spreading factor, but this is not necessarily the case. Conversely a hemolysin-free filtrate may show pronounced spreading.

2. An antitoxic serum, which in experiments in vivo neutralises the dermonécrotic toxin, has no influence on Duran-Reynals spreading factor. Nor is there any effect of serum from a highly allergic rabbit. However it appeared that an antibacterial serum had a pronounced inhibiting effect on the spreading factor.

3. These experiments, which show that the antibacterial serum in vivo can prevent spreading in a manner in which the antitoxic serum cannot, indicate that there are other antibodies than the antitoxic ones which play an important part in staphylococcal infections.

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THE BACTERICIDAL AND GROWTH-INHIBITING EFFECT OF POLYTHIOSOL AND EUTHIOSOL ON STAPHYLOCOCCUS AUREUS

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(Received for publication 10th Dec. 1943).

In the treatment of acne vulgaris and seborrhoea good effects have been observed during the past year or two from two special preparations, »Euthiosol« and »Polythiosol«, made by Swoga chemical work, Ltd., Copenhagen (5).

The remedies contain various substances which may be credited with having therapeutical effects, i. a. colloidal sulphur and polythionic acids, and the present investigation was performed in order to find out which of the components are active on the bacteria of the skin when the remedy is being employed. For the investigation we elected to use a *Staphylococcus aureus*, isolated from an acne vulgaris patient.

An analysis of the two preparations, made according to A. D. Mitchell and A. M. Ward (4) gave the following result:

	Polythiosol		Euthiosol	
	Millimol/ litre	Milligram/ litre	Millimol/ litre	Milligram litre
Sulph. hydrogen (H_2S).....	0	0	3.2	109
Sulphite (SO_3^-).....	0.48	38	0	0
Thiosulphate ($S_2O_3^-$).....	0.30	34	1.8	202
Trithionic acid ($S_3O_6^-$).....	0.50	96	10.3	1980
Tetrathionic acid ($S_4O_6^-$).....	32.2	7210	2.4	538
Pentathionic acid ($S_5O_6^-$).....	35.1	8990	0	0
Colloidal sulphur	0.03%		7%	
pH.....	0.7		2.1	

The investigation is in two sections: bactericidal effect and growth inhibition.

A. Bactericidal Effect.

a. Experimental Technique.

In *Zeitschrift für Immunitätsforschung und experimentelle Therapie* 101 Band, page 61, Ivanovics describes a nephelometric method for determining the bactericidal potency of chemotherapeutica. Having no nephelometer we have been unable to employ this method, and therefore we have made use of a procedure as described below. The nutrient medium employed for cultivation is a beef broth prepared according to Levine & Schoenlein (2).

Of the broth 5 ml. is transferred by pipette to a test-tube, which is stoppered with cotton-wool and koched for 10 minutes. After cooling the tubes are placed in the incubator at 37° for 24 hours in order to control their sterility.

One of the tubes is inoculated with a pure culture of *Staphylococcus aur.* by means of a loop, of which the eye is shaped over a match. The tube containing the pure culture is held aslant and the eye of the loop inserted parallel with the surface of the fluid. By this means the loop picks up a lentil-shaped drop of fairly constant size. After inoculation the tube stands at 37° C. for about 24 hours, whereafter the number of bacteria is very constant. To 10 ml. of sterile salt solution (0.9 %) in a test-tube we now add the preparation whose bactericidal effect it is desired to test, followed by 0.1 ml. of the aforesaid bacterium culture, mix well and inoculate from it with the loop at fixed intervals into tubes containing beef broth. These tubes are allowed to stand during the night at 37°, whereafter one can see in which of the tubes there is any growth. In order to make doubly sure the tubes stand at 37° for another 24 hours, and the first clear (i. e. sterile) tube in the row thus marks the time elapsing until the bacteria in the salt solution are killed. It may be said here and now that the line of demarcation between unsterile and sterile tubes has been quite sharp in all the experiments described below. In no case did any cloudy (i. e. unsterile) tube appear in the row after the first clear one. This presumably is due to the technique with the relatively long time between each inoculation (from 15 minutes to 2 hours). We were persuaded by this sharp line not to set up the inoculation results in tables, but to use the first sterile tube as a measure of the bactericidal effect arrived at. This saves a good deal of space.

In the following experiments the inoculations were made at intervals of 0 — $\frac{1}{4}$ — $\frac{1}{2}$ — 1 — 2 — 4 — 6 — 8 hours.

b. Acid Effect.

In view of the low pH of polythiosol it was natural to ascertain what rôle was played by the hydrogen-ion concentration itself, as

acids are known to be antiseptic. The salt solutions were therefore prepared with different pH, and the following lethal times were then found:

Experiment 1.

Acid effect.

Ml. polythiosol to 10 ml. salt solution	pH	Lethal time, in hours
0.2	2.4	$\frac{1}{2}$
0.2	5.4	>7
0.2	6.7	>7

The polythiosol concentration being the same, the difference in the bactericidal effect may be due either to the change in the pH or, as will be seen from the following, the consequent displacement of the quantitative ratio between tetra- and pentathionic acid. An analysis of polythiosol after 24 hours at the above pH values gave the following result:

pH	Millimol per litre	
	$S_4\bar{O}_6$	$S_5\bar{O}_6$
2.4	30	39
5.4	50	25
6.7	52	25

This shows that increasing pH displaces the quantity of $S_5\bar{O}_6$ as against $S_4\bar{O}_6$; as will be shown later, however, this has no influence (or at any rate no influence that is measurable by this method) on the bactericidal effect in comparison with the hydrogen-ion effect itself.

In order to ascertain whether the antiseptic effect depends on the pH alone the following experiment was made:

Experiment 2.

Ml. sulphuric acid to 10 ml. salt solution	pH	Lethal time, in hours
0.3 ml. n/1 sulph. acid	1.7	$\frac{1}{2}$
0.3 " n/10 "	2.8	>24
0.3 " n/100 "	5.7	>24
0.3 " n/1000 "	6.5	>24

This shows that sulphuric acid must be brought down to a pH

of 1.7 in order to have the same effect as polythionic acids at a pH of 2.4.

The following experiment was made for the purpose of determining more exactly the pH at which the antiseptic effect of the acid begins to appear:

Experiment 3.

The bactericidal effect of sulphuric acid solutions with a pH between 1 and 3. The same quantities of bacterium culture and common salt as in the foregoing experiments are added to a number of sulphuric acid solutions.

Tube No.	pH	Lethal time, in hours
1	1.09	0
2	1.64	$\frac{1}{6}$
3	2.06	$\frac{1}{2}$
4	2.53	6
5	2.94	>24

The result is plotted in Fig. 1 A, which shows that with a pH = 2.5 the bacteria have a very short existence; above pH = 3 some at any rate have a very long lifetime.

In order to exclude the possibility of this bactericidal effect being due to a specific sulphuric acid action, and not solely to the low pH, Experiment 3 was repeated, with the difference that the sulphuric acid solutions were replaced by citric-acid-phosphate buffer solutions, and the common salt was omitted.

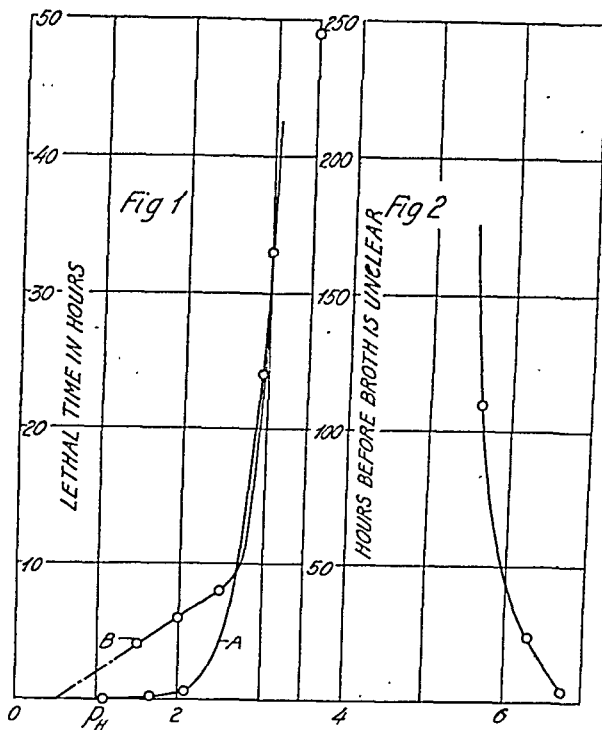
Experiment 4.

Bactericidal Effect of Buffer Solutions.

Tube No.	pH	Lethal time, in hours
1	1.5	4
2	2.0	6
3	2.5	8
4	3.0	33
5	3.5	>49

The result is reproduced graphically in Fig. 1 B. In the vicinity of pH = 3 the curves run parallel. Whether or not the deviation under pH = 2.6 is due to the intensification of the acid effect by the common salt content in A (see Fig. 1) has not been examined.

Comparative tests of the effects of various common acids were made in Experiment 5 for the purpose of obtaining additional verification.



Experiment 5.

The bactericidal effect being so greatly dependent on the pH, we have in the following endeavoured to keep the pH constant (by the addition of buffer solution instead of salt solution) — when investigation the bactericidal effect of various substances.

Tupe No.	Contents	pH	Lethal time, in hours
1	5 ml. n/100 sulph. acid + 5 ml. buffer sol.	2.15	8
2	» Hydroch. acid + »	2.17	10
3	» nitric acid + »	2.14	10
4	» phosph. acid + »	2.16	8
5	» citric acid + »	2.17	8
6	Buffer	2.17	10

The effects of the acids are practically equal.

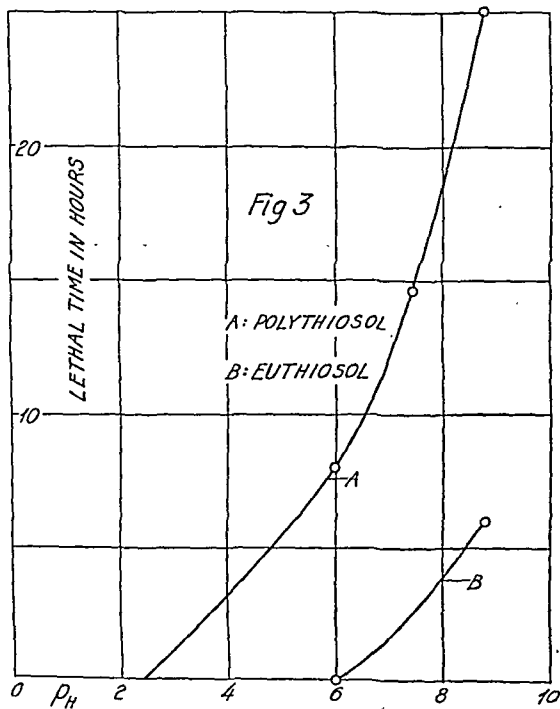
At pH = 2.5 the two preparations have the same effect, but at pH values higher than 3 Euthiosol is more potent than Polythiosol (see Fig. 3). As regards the latter it is probable that the principal effect is a hydrogen-ion action supported by the other components, whose actions will appear from Experiment 7. The fact that the bactericidal effect of Polythiosol is almost nil at pH values around the neutral point agrees with investigations by O. N. Liming (3),

Experiment 6.

Comparison of the bactericidal effect of Polythiosol and Euthiosol with different pH values.

ml. antiseptic to 10 ml. buffer solution	pH	Lethal time, in hours
0 ml. (buffer solution).....	2.50	> 7
0.3 » Polythiosol.....	2.46	$\frac{1}{2}$
0.3 » Euthiosol.....	2.49	$\frac{1}{2}$
1 » Polythiosol.....	3.0	1
1 » Euthiosol.....	3.0	0
1 » Polythiosol.....	6.0	> 8
1 » Euthiosol.....	6.0	0
1 » Polythiosol.....	8.8	>25
1 » Euthiosol.....	8.8	6

who demonstrated that pentathionic acid is active only in acid solutions. At pH = 3 and 6 Euthiosol is instantaneously lethal. At pH = 8.8 there was no lethal effect until after 6 hours, which is connected with the circumstance that the sulphur sol had begun to coagulate at this pH value.



c. Other Active Components.

Experiment 7.

In order to find out how great a share the thionic acids, sulphite, sulphuretted hydrogen and thiosulphate have in the bactericidal effect of Polythiosol and Euthiosol respectively, buffer solutions were prepared with corresponding quantities of these components and compared bactericidally with Polythiosol and Euthiosol.

Ml. antiseptic to 10 ml. buffer solution	pH	Lethal time, in hours
0.3 ml. Polythiosol.....	2.37	1
Sulphite solution.....	2.40	6
Sulph. hydrogen sol.....	2.40	6
Thiosulphate sol.....	2.31	>8
Buffer solution.....	2.44	>8

At pH = 2.8 and pH = 5.8 a comparison has been made between Polythiosol and a 0.2 m tetrathionic acid both undiluted and diluted in such a manner that the tetrathionic acid content was just as high as the sum of tetra- and pentathionic acids in Polythiosol.

ml. antiseptic to 10 ml. buffer solution	pH		Lethal time, in hours	
	I	II	I	II
0.3 ml. Polythiosol.....	2.87	5.78	1	>21
0.3 „ H ₂ S ₄ O ₆ undiluted.....	2.80	5.73	2	>21
0.3 „ „ diluted.....	2.85	5.78	4	>21
Buffer solution.....	2.88	5.84	6	>21

As was anticipated, tetrathionic acid, sulphite and sulphuretted hydrogen intensify the acid effect, but individually they have a weaker action than Polythiosol. If the analyses of Polythiosol and Euthiosol are considered in conjunction with the results of these experiments it becomes evident that there are at any rate two possible causes of the stronger bactericidal effect of Euthiosol:

- 1) The high content of trithionic acid in Euthiosol,
- 2) „ „ „ „ colloidal sulphur in Euthiosol.

In order to test the first possibility Polythiosol, which contains much tetra- and pentathionic acid, was treated with sulphite, whereby the tetra- and pentathionic acids were reduced to trithionic acid. Of this reduced Polythiosol, which contains much more trithionic acid

(about 6.5 times as much) than Euthiosol, 1 ml. was added to 10 ml. buffer solution, the result being:

$$\text{pH} = 6.0$$

$$\text{Lethal time} > 8 \text{ hours,}$$

that is to say an effect much inferior to that of Euthiosol, which in the same concentration and at the same pH kills bacteria instantaneously (see Experiment 6).

d. Effect of Colloidal Sulphur.

I. Polythiosol.

Experiment 8.

ml. antiseptic to 10 ml. salt or buffer solution	pH	Lethal time, in hours
0.3 ml. Polythiosol.....	2.63	4
0.3 „ „	2.70	3
0.3 „ „ , freed of colloidal sulphur..	2.45	2
0.3 „ „ , freed of colloidal sulphur..	2.77	5
1.0 „ „	6.3	>8
1.0 „ „ , freed of colloidal sulphur..	6.0	>8

Within the pH limits investigated we did not succeed in demonstrating any antiseptic difference in Polythiosol *with* and *without* colloidal sulphur.

II. Euthiosol.

Experiment 9.

In order to ascertain what part is played by the sulphur. sol. in Euthiosol it was removed in the following two ways:

1. Adding sulphuretted hydrogen and filtering off the coagulate.

2. Adding 1% NaCl and filtering off the coagulate.

Of the filtrate 2 ml. was added to 10 ml. buffer solution and, for comparison, 2 ml. Euthiosol to 10 ml. buffer solution.

	pH	Lethal time, in hours
Euthiosol	6.0	0
„ , precipitated with H ₂ S	6.0	>8
„ „ „ NaCl	6.0	>8

The result suggests that the effect of Euthiosol at this pH is due mainly to the sulphur sol. At the original pH (2.1) there is simultaneously an acid action (see Fig. 1). From an article by Prica, M. i. Zeitschr. f. Hyg. (6) it appears that other substances act in a similar manner. When fine-grain shore sand is allowed to stand for some months with distilled water, the solution acquires a strong bactericidal effect on *B. coli*, paratyphi B and *Staph. pyogenes aureus*. The action is ascribed to the formation of colloidal silicic acid in a concentration of about 0.073 g. per litre. If the colloidal silicic acid is removed the bactericidal effect is lost. Similar observations were made with colloidal solutions of aluminium and ferri-hydroxyde.

In order to ascertain whether the bactericidal effect of Euthiosol is due to the sulphur sol being absorbed by the bacteria and inhibiting their growth, or to a direct lethal action, we instituted Experiment 10, in which at certain intervals we added common salt to coagulate the sol, whereby we should learn whether the bacteria are freed and continue to survive after the coagulation.

Experiment 10.

To four test-tubes each containing 10 ml. buffer solution (pH=5.5) are added 1 ml. Euthiosol and 0.1 ml. bacterium culture, whereupon inoculations are made immediately from all four tubes to broth. This completed, 1 % NaCl was added at once to Tube No. I. After 15 minutes inoculation was made to broth from all four tubes and 1 % NaCl was added to Tube No. II, and so on, with inoculations at the times shown below.

No.	After time	Salt added	Tube Nr.			
			I	II	III	IV
1	0'	I	+	+	+	+
2	15'	II	—	—	+	—
3	30'	III	—	—	—	—
4	45'	IV	—	—	—	—
5	1 hr.		—	—	—	—
6	1½ hrs.		—	—	—	—
71	2 hrs.		—	—	—	—

The experiments indicate that there is direct lethal action. The control solution with 5 % NaCl had growth throughout the experimental period. The experiment was repeated with the same result with water instead of buffer solution, and the salt addition was 5 %.

Experiment 11.

In conclusion, a test was made of the bactericidal effect of Euthiosol using a broth-buffer solution of pH=6 with the same concentration of bacteria as above.

ml. Euthiosol to 10 ml. solution	pH	Lethal time, in hours
0.1 ml.	6.3	>8
0.3 »	6.3	0
0.6 »	6.3	0
1.0 »	6.3	0
1.0 » } without sulphur sol.	5.8	>8
1.0 » }	5.8	>8
1.0 » plus 5 % NaCl	5.8	0

This experiment provides additional confirmation of the assumption that the precipitation of the colloidal sulphur in Euthiosol causes it to lose its bactericidal effect. The final test, in which 5 % NaCl was added to the Euthiosol-broth solution, was immediately lethal; the presence of the beef broth inhibits very considerably the coagulation of the sulphur sol, a fact which we have checked by means of a number of salting-out tests.

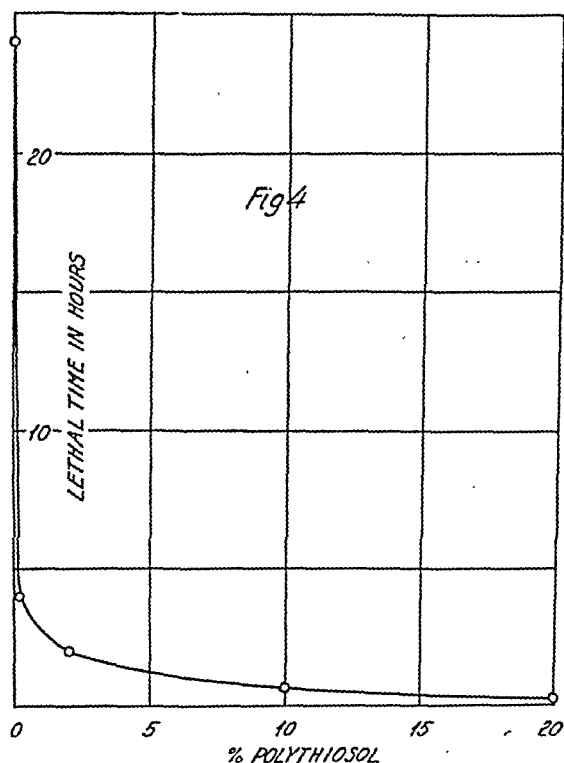
The experiment tells us nothing directly about the cause of the action of colloidal sulphur, but it is inferable that it is not a matter of growth inhibition, for in that case there would have been growth in Experiment 10 after the coagulation of the sulphur sol. The results suggest that there is direct lethal action.

Experiment 12.

In this experiment we examined the influence of the Polythiosol concentration on the bactericidal effect.

Concentration	pH	Lethal time, in hours
40% Polythiosol	2.48	0
20% »	2.43	$\frac{1}{6}$
10% »	2.46	$\frac{2}{3}$
2% »	2.27	2
0.2% »	2.30	4
0.04% »	2.30	6
0.02% »	2.32	10
Buffer solution	2.37	24

These results are reproduced graphically in Fig. 4. The curve describes a hyperbola of the same type as that described by Madsen & Nyman (1).



B. Growth Inhibition.

As a supplement to the foregoing and in additional confirmation of the results we made some few tests for the purpose of examining the growth-inhibiting effects of Polythiosol and Euthiosol.

In order to save space we omit to report the experiments in extenso and are content to give the results and a description of our procedure.

In test-tubes we made a mixture of 5 ml. broth and 5 ml. buffer solution, and the tubes were koched for about 20 minutes. To the mixture was added a thoroughly shaken 24 hour bacterium culture diluted so that the final solution had a dilution degree of 10^{-5} . This gives a workable dilution so that the various colonies can be counted, and the tubes were placed in the incubator at 37° C. At suitable intervals smears were made on agar plates with a platinum loop. After inoculation the plates were kept at 37° for 16 hours, whereafter the colonies were counted.

It is also possible to obtain a direct estimate of the growth inhibition by watching to see if growth occurs in the tubes containing the broth buffer solution.

Both the tubes containing broth buffer solution and counting on

Agar plates indicated growth inhibition between pH 4.9 and 5.6 (Fig. 2).

In the following experiments we examined the action of Polythiosol and Euthiosol in dilutions varying from 1 : 1 to 1 : 1000 at a pH = 7. For Polythiosol there was no plain inhibition at all while Euthiosol diluted 1 : 1 is strongly bactericidal at pH = 7 in accordance with Experiment 6. Diluted 1 : 10 and 1 : 1000 Euthiosol had no more inhibitory effect. At pH = 6.3 we found that Polythiosol 1 : 1 and 1 : 10 inhibited the growth of the bacteria whereas Euthiosol 1 : 1 killed them instantaneously.

In our last experiment we compared Polythiosol and Euthiosol (diluted 1 : 1) with a solution of sulphite with the same concentration as Polythiosol and a solution of sulphuretted hydrogen with the same sulphuretted hydrogen content as Euthiosol at pH = 6.3. We stated hereby that subphuretted hydrogen alone has an inhibitive effect just as strong as Polythiosol 1 : 1 at pH = 6.3.

Summary.

The results of the experiments may be recapitulated as follows:

1. The main action of Polythiosol is an acid action that is supported by the other components of the remedy.
2. The main action of Euthiosol originates from the sulphur sol and is supported by a weak acid action and by the polythionic acids and sulphuretted hydrogen.
3. The effective scope of Euthiosol within the pH-scale is consequently much greater than that of Polythiosol.
4. The acids have the same effect regardless of the anion.
5. Growth inhibition like bactericidal effect is greatly dependent on the pH and lies at a pH value between 4.9 and 5.6. At lower than pH 3 the bacteria are killed quickly, lower than pH 1 instantaneously.

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EARLY CHANGES IN THE LIVER IN EXPERIMENTAL HYPERTHYROIDISM IN RODENTS

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(Received for publication December 22nd, 1943.

Since *Rössle's* publication in 1933 the pathological changes in the liver of exophthalmic goiter patients have been the object of particular interest. He described acute and chronic changes. The former consisted mainly of changes in the blood capillaries with pronounced morphological changes which contributed to the development of pericapillary oedema, which was in turn regarded as a factor in serous hepatitis. Moreover, he described degenerative changes in the liver cells, most frequently in the form of slight liver cell atrophy, but also more severe changes which correspond to the picture in an acute or sub-acute yellow liver atrophy. *Rössle* laid most emphasis on the capillary affection and regarded the vessels as the primary object of the effect of thyroxin on the liver.

The chronic changes were characterized by a peculiar sclerosing process, which will not be described in detail in the present paper. In the same year *Johan Holst* called attention to the frequent occurrence of pathological liver changes in thyrotoxicosis.

Rössle's interpretation of the pathogenesis of the liver in exophthalmic goiter patients has awakened lively discussion. It is supported by a number of clinical investigators (*Haban* 1933—35, *Eppinger* 1935). Others explain the primary cause as a toxic effect on the liver epithelium without, or combined with, cardiac stasis.

The finding that experimentally-produced hyperthyroidism in animals showed liver changes which in many ways resembled those seen in clinical exophthalmic goiter material gave stimulus to a number of investigations in this field. *Gerlei* (1933) injected 4—8 mg. thyroxin subcutaneously in rabbits. The animals lost weight rapidly and died spontaneously after 5—7 days. On histological examination of the liver he found centrilobular necrosis of the liver cells and hyperæmia. *Haban* (1935) injected rabbits, rats and guinea-pigs subcutaneously

with thyroxin. On the rabbits he used total doses of 10 to 570 mg. The animals died spontaneously after from 4 to 48 days. He demonstrated dilated capillaries with pericapillary oedema and in 4 rabbits which died of intercurrent diseases he found indications of extreme destruction of liver cells. He assumed that there was a primary toxic effect on the capillary walls, while the injury to the liver cells was due to the combined effect of intercurrent disease and thyroxin.

Manzini and co-workers (1935 and 1936) demonstrated that after the subcutaneous injection of 2—4 mg. thyroxin daily in rabbits there appeared extensive necroses of the liver tissue and in addition considerable dilation of the vessels. Further investigation with vital staining and histamin injection failed to reveal morphological changes in the capillary walls.

Schönholzer (1936) studied a material consisting of 24 rats with total doses from 0.5—6.5 mg. thyroxin. After an observation period from 7 to 20 days the animals were killed, he found capillary dilation but interpreted it as an expression of atrophy of the liver cells. He found no other circulatory disturbances or changes in the liver cells with the exception of reduction of glycogen and a lack of capacity to deposit albumin substances (determined by control of intracellular albumin granules stained with methyl green pyronin). In a later publication (1937) where he used thyrotropic hypophysis hormone he found principally the same changes.

Heinlein and *Dickhoff* (1936) demonstrated considerable circulation disturbances in the liver of chronically thyroxin-intoxicated cats. These consisted of pericapillary oedema and small hemorrhages in the substance. The liver cells showed some fatty degeneration. They concluded that thyroxin was an angiotropic toxin which did not have a specific effect on the liver but perhaps principally on the heart.

Sciaky (1938) examined in guinea-pigs, rabbits, cats and rats, in all 18 animals, the effect of an acute intoxication with strong doses of thyroxin observed over a period of 8—10 days. He also examined animals chronically intoxicated with weak doses after an observation period of 77—196 days. The animals died spontaneously. He demonstrated degenerative changes in the vessels, especially the central vein, as well as pericapillary oedema. He assumed that the first lesions in the liver were caused by the changes in the vessels, but he also believed that there was a cardinal stasis of pathogenic significance in addition to the toxic factor, as the lesions could not be regarded as specific for thyroxin.

In 1938 *Kastert* examined 16 rats with an average weight of 90—100 g. They were given thyroxin subcutaneously in single doses of 0.5 mg., the largest total dose was 4.5 mg. Observation period 2—32 days after which the animals were killed. Glycogen in the liver disappeared after 2 mg., albumin granules after 4.5 mg. He found necrosis of the liver cells in only one animal but found necrosis of

the liver cells in only one animal but found necrosis often in the control material where there was a *Salmonella* infection.

Zeldenrust and *van Beek* (1939) examined liver changes in 16 rabbits which had been injected subcutaneously with 0.2—1 mg. thyroxin daily with irregular dosage. They died in the course of 44—302 days after total doses of 15—83 mg. thyroxin. They demonstrated liver cell atrophy and frequent cell necrosis. They explained capillary dilation, which was a constant finding, as a result of cell atrophy and cardiac stasis. They found no clear indications of serous hepatitis.

Ström (1942) examined 17 rabbits distributed in 3 groups after subcutaneous thyroxin injection. One group of 4 animals, which had been given a single dose of 10 mg. and were killed after one hour, showed no pathological liver changes. Another group of 9 animals were given 5 mg. daily with an observation time ranging from 7 to 16 days. These showed a diffuse liver cell atrophy and marked dilation on the capillaries. He interpreted the liver cell atrophy as the primary morphological change and the changes in the vessels as a secondary result.

Material and Methods.

The material consisted of rabbits and rats, all males. A total of 30 rabbits were used in the experiments and in addition 12 normal controls. The total number of rats was 10 as well as some normal controls. Most of the rabbit material, in all 23 were injected subcutaneously with thyroxin, 2 cc 1‰. The remaining 7 were given elityran subcutaneously because of lack of thyroxin. The rat material, which consists of the laboratory's strain of albino rats, was injected with elityran.

The animal material is much smaller than originally intended, due to the lack of thyroid preparations. However since it is the early changes which have been the object of our investigations and the experimental sequence in this respect may be considered satisfactory, they should afford the basis for reliable conclusions. We therefore feel justified in publishing these experiments, the more so because the methods employed deviate from those used previously in similar investigations.

The experimental material is divided into 5 groups according to dosage and animal species. As far as the findings are concerned, these are naturally grouped around the largest rabbit group (Group I) and must therefore be regarded as a supplement to the latter. The animals were given one injection daily. Those with a longer observation time than 24 hours were killed 24 hours after the last injection.

The weight of the rabbits at the beginning of the experiments was generally about 1500 g. In the course of the experiments they have lost an average of about 31 % in weight. Their general condition

in the case of the larger doses was rather poor towards the end and they ate but little. In some instances we have therefore had normal controls, which were given the same diet during the same observation period in order to exclude the possibility that the pathological findings in the liver could be a result of deficient nourishment. The rats weighed on the average about 250 g. at the beginning. Their weight curve falls sharply at the beginning of the experiments, but later there is a slight increase so that some of the rats at the conclusion of the experiment had almost attained their original weight. They lost an average of 13 % in weight.

In order to examine the capillary functions in the liver, most of the animals were injected intravenously with hydrocollag solution or 5 % trypan blue solution or a mixture of both. In previous investigations trypan blue has proved to have a special affinity for serum albumins. It was our hope that this relation might be of help in the study of the pericapillary oedema on the theoretic assumption that trypan blue, which in the blood is linked to the serum albumins, would follow these on diffusion in the pericapillary spaces and possibly stain them a pronounced blue. However, the serum albumin concentration differs so little in intracapillary blood and in the pericapillary oedema fluid that the difference in color intensity is relatively small. However, as will be described below, trypan blue has been very useful in judging the permeability relations in capillary walls and liver cell membranes. Moreover, it has proved to have a strong affinity to the reticular fibrillae which lie close to the capillary walls as well as the oedematous capillary wall, an observation which does not appear to be previously recorded in the literature. Neither trypan blue nor hydrocollag appear to have any contributory effect on the liver, either histologically or histochemically when injected intravenously into normal controls with the dosage employed.*) Even though the application of these foreign substances may to a certain extent be regarded as a complication in the experiments, it may be assumed that they do not cause pathological changes in the liver which might lead to misinterpretation of the histological findings. In some animals, where there has been the least suspicion of secondary infectious processes, the organs have been examined bacteriologically with negative results.

The histological preparations were fixed with 10 % formalin. Susa-Heidenhain's fluid, Carnoy's fluid, Müller-formol, absolute alcohol. Champy's and Regaud's fluids. The preparations are stained with hematoxylin-eosin in the ordinary manner. Moreover, special staining has been carried out for fat, glycogen, argentophile fibrils and mito-

*) In a series of animals a liver biopsy was taken before the injections which also showed that no artificial changes result from this method.

chondria. Heidenhain's iron hematoxylin method was used for special examination of the nuclei.

Results.

In the following description each of the 4 tissue components of the liver which have been specially examined will be discussed individually. These are blood vessels, liver cells, supporting tissue and reticulo-endothelial tissue.

Animal Group I.

This group consists of 13 animals which were daily injected with 2 mg. thyroxin, with total doses varying from 2 to 22 mg. The doses were 2, 4, 6, 8, 10, 12, 14, 18, 20 (4 animals) and 22 mg.

1. *Changes in the blood vessels.* These are indicated as early as after the first dose of 2 mg. but are not clear until after doses of 4—6 mg. The first indication is a moderate dilation of the liver sinusoids. This is first visible in the peripheral part of the lobes (Fig. 1), more rarely intermedially. Only in very rare instances is there a marked centrolobular dilation, a finding which is in disagreement with that of several other investigators and which indicates that the

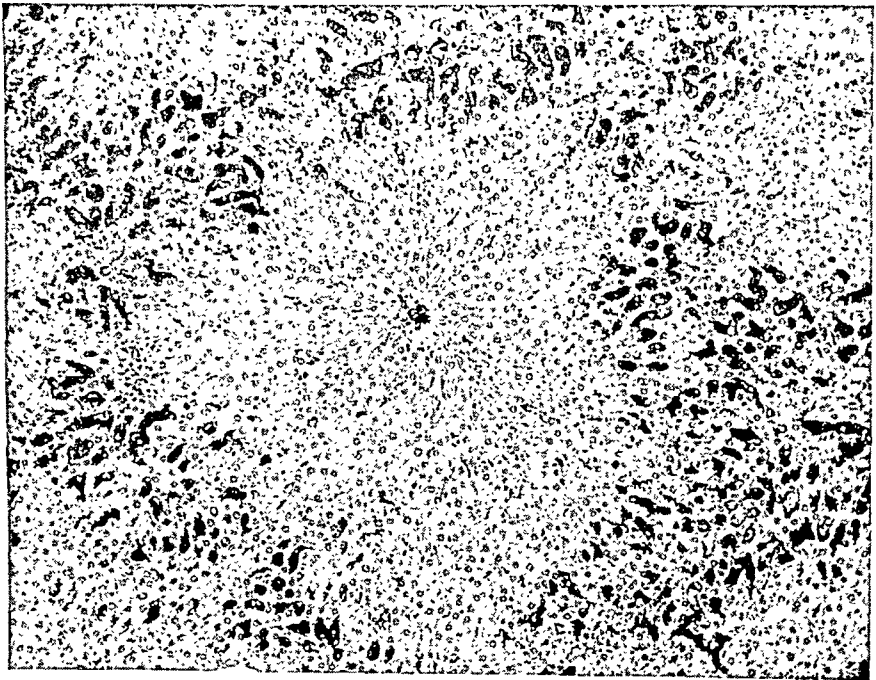


Fig. 1.

× 150. Rabbit No. 31, thyroxin dose 2 mg., observation 1 day. India ink injected. Dilation of liver capillaries peripherally in the lobuli.

dilatation is not due to a passive venous stasis. Another finding which also indicates that the dilation is not due to a venous stasis is that the dilated sinusoids contain few erythrocytes. Nor is there any indication of conglutination of red corpuscles or other signs which are characteristic of a true stasis. Even though ordinary histological

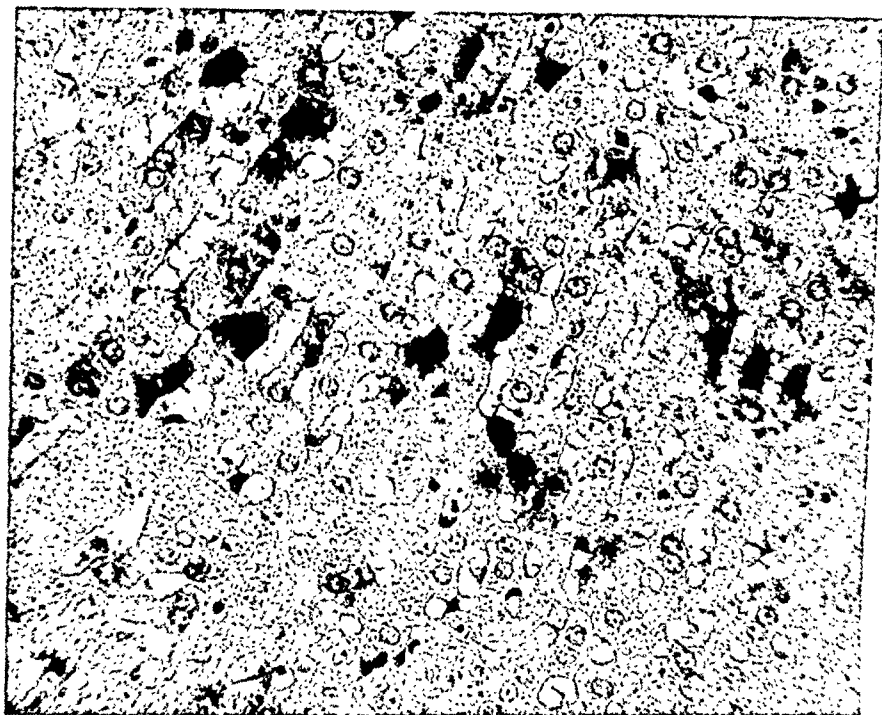


Fig. 2.

× 350. Rabbit No. 29, thyroxin dose 6 mg., observation 3 days. Scattered liver cells have absorbed trypan blue.

methods do not reveal any other signs of circulatory disturbance than this slight capillary dilatation, our physiological capillary tests seem to indicate that there are also permeability disturbances in the capillary wall, although these cannot be regarded as a consequence of dilation (cf. Krogh's investigations). When animals are injected intravenously with trypan blue, this permeability disturbance is revealed by the fact that individual liver cells, both nucleus and cytoplasm, take on a strong blue color. This blue staining seems to be locally limited after small thyroxin doses, often monocellular (Fig. 2). These blue cells always lie close to the walls of dilated sinusoids. Further comments on this finding will be made under the section on changes in the liver cells. Here it suffices to remark that we have interpreted it as a combined effect of increased local permeability of the vessel wall and a beginning degeneration of the liver cell in question. We have not demonstrated any increased permeability for

larger particles such as hydrocollag after small doses. Nor have we found any histological changes in the vessel walls, either of progressive or regressive nature.

On increasing doses of thyroxin there is a marked increase in changes in the vessels. There seems to be a proportionate difficulty

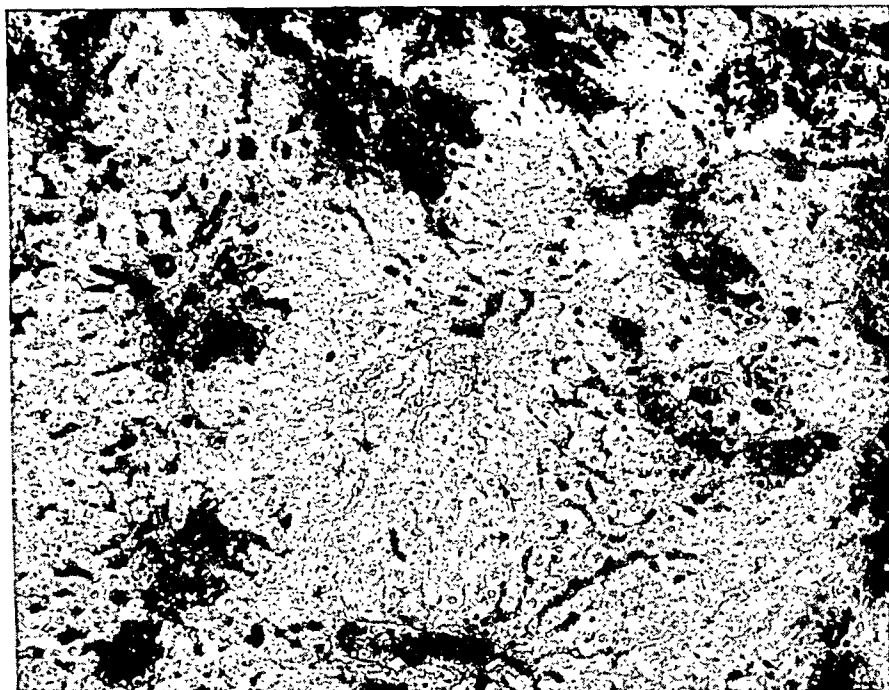


Fig. 3.

× 250. Rabbit No. 15, thyroxin dose 12 mg., observation 6 days. Accumulation of injected hydrokollag in peripheral liver sinusoids.

for blood circulation in the sinusoids. After 10—12 mg. thyroxin it is seen in hydrocollag-injected preparations that the particles remain in the distended sinusoids (Fig. 3), densest peripherally, even though the capillary dilatation extends throughout the entire lobulus. It is not seldom seen that the dilated sinusoids perilobularly may be almost congested with hydrocollag particles, an indication that there has been practically no blood supply. Judging by this particle stagnation, it seems there is an almost complete blockade of circulation of corpuscular elements in the blood. After the same doses there are also anatomical and more diffuse histochemical changes in the walls of the sinusoids. These are expressed as a thickening of the walls of the vessels, which, in addition, in trypan blue-stained preparations, are more deeply stained than usual. In addition there is often a thickening and deeper staining and sometimes fraying of pericapillary reticular fibrils, possibly the first indication of their proliferation (Fig. 4).



Fig. 4.

× 1000. Rabbit No. 9, thyroxin dose 14 mg., observation 8 days. Trypan blue injected. Pericapillary reticular fibrils.

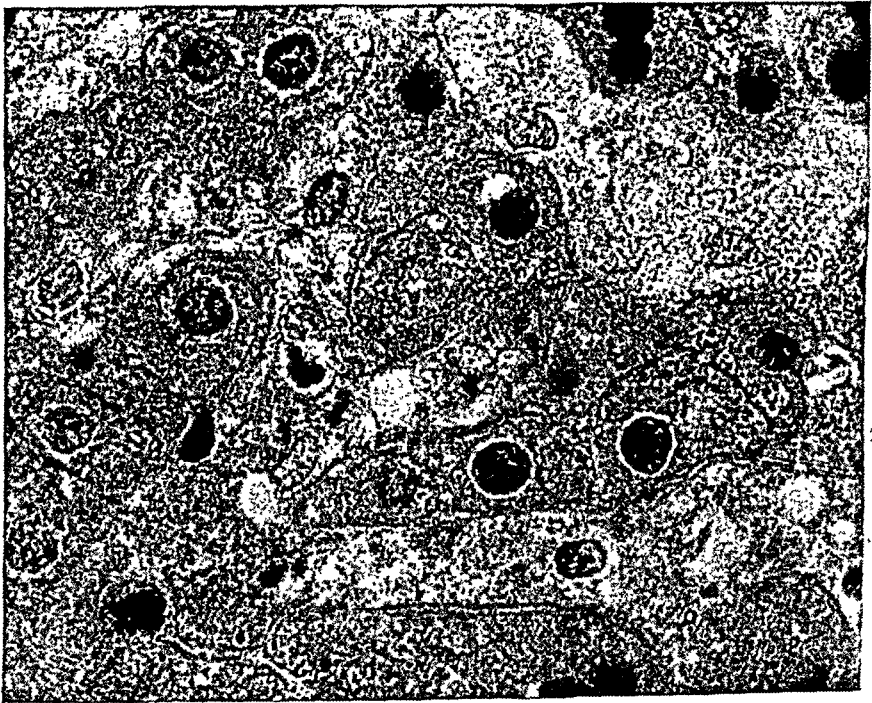


Fig. 5.

× 1000. Rabbit No. 17, thyroxin dose 18 mg., observation 9 days. Intramural oedema.

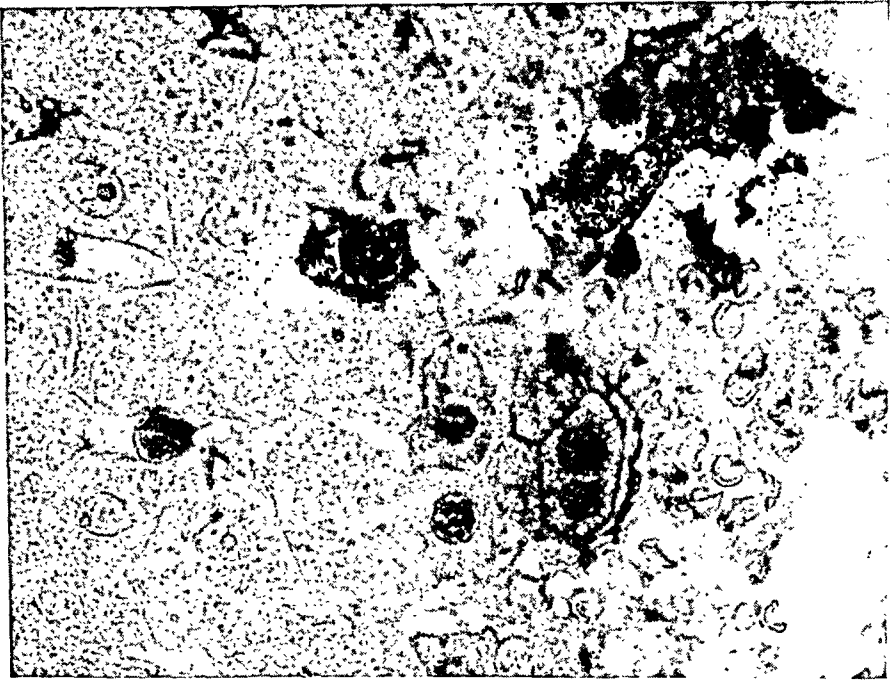


Fig. 6.

× 1000. Rabbit No. 14, thyroxin dose 22 mg., observation 11 days.
Pericapillary oedema.

The lining cells in the sinusoids, however, seem to be comparatively resistant and show no regular degenerative changes either after these doses or the largest total doses within this animal group. We have interpreted the described changes in the vessel walls as an intramural oedema (Fig. 5), a capillary oedema. Pericapillary oedema, which is described by numerous investigators as the primary vascular changes in the liver in hyperthyrosis and a number of other intoxications; may be a more advanced developmental stage of this capillary oedema. Pronounced pericapillary oedema and shedding of the coating cells of the capillaries have been demonstrated after the largest doses in this series (Fig. 6). However, these seem to be only local changes and they in no way dominate the morphological picture of circulatory disturbances in the case of the moderate thyroxin doses which we have employed. Severe circulatory disturbances with extravasation of red blood corpuscles or larger particles (hydrocollag) have not been observed in this material.

2. Liver cell changes. The first changes which we have observed in the liver cells appear after thyroxin doses of 6 mg. and are, in a way, functionally histological. They are apparently not previously discussed in the literature. They appear immediately after the first changes in the vessels which are described above, but are of a less

pronounced nature than these primary findings. They find expression in trypan blue-injected animals as mentioned above, in the form of blue-stained individual liver cells. These cells are found in the walls of dilated liver sinusoids and therefore preferentially peripherally or intermedially in the lobe to begin with (Fig. 7). They thus

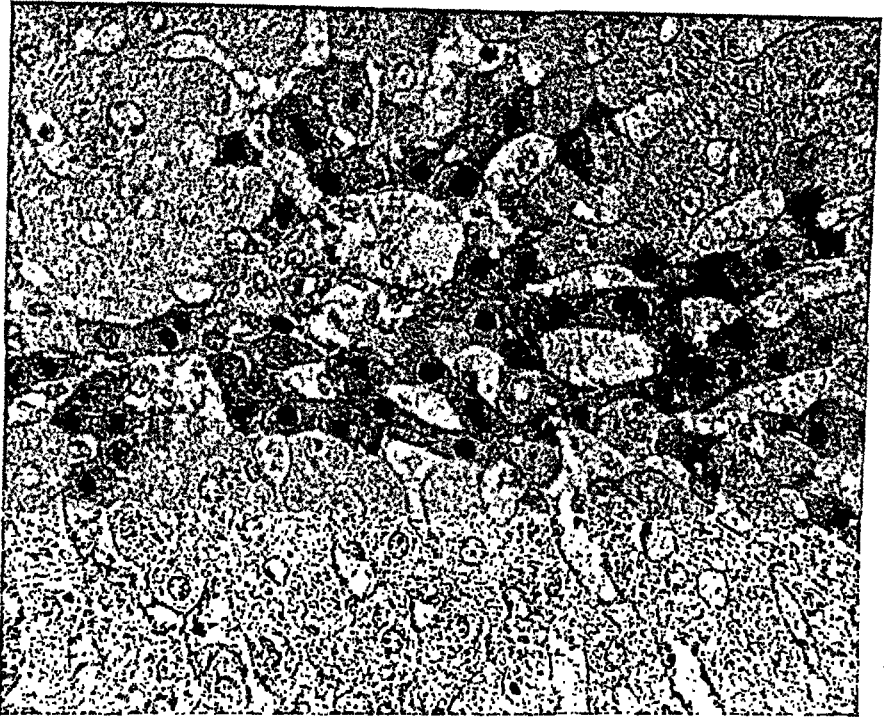


Fig. 7.

× 600. Rabbit No. 15, thyroxin dose 12 mg., observation 6 days. Trypan blue stained liver cells around dilated capillaries.

arise in the same places as the primary vascular changes. This blue staining is diffuse and includes both cytoplasm and nucleus. After larger doses they are found more extensively in the lobulus and not infrequently along the larger veins. This blue staining of the liver cells has not been observed in normal control animals nor in animals with starvation livers. It is distinguished from active cellular deposit which is granular and which requires at least 8—10 hours after the injection of stain before it is visible. Thus it cannot be assumed to be a result of active phagocytotic cell function. These blue-stained cells exhibit no histological characteristics of cell degeneration either in the nucleus or cytoplasm. Cells with clear regressive changes do not show this staining so characteristically.

The mechanism of this blue staining may be interpreted as an increased permeability for relatively small colloidal dyestuffs in the structural formation, which consists of the vessel wall and the mem-

brane in a corresponding place in the liver. This is, however, an anatomical block which, as far as the liver is concerned, is not sufficiently elucidated. Most investigators of the anatomy of the liver are agreed that the sinusoid walls differ from other capillary walls in that they have no anatomical continuity. This theory is supported from a purely theoretical viewpoint since it is known that the Kupffer cells comprise a part of the covering cells and further that these cells are highly labile as to form and size and ability to loosen from the underlying tissue. The possibility which our investigations indicate must be borne in mind, namely that liver cell membrane may in some cases form a part of the vessel wall. In such a case this blue staining of individual liver cells does not necessarily signify an increased permeability in the vessel wall itself, but only in the liver cell membrane. As both the nucleus and the cytoplasm take the blue stain there must also be abnormal permeability relations for the nuclear membrane. It has previously been demonstrated by other investigators that a normal cell nucleus does not take vital stain, but only nuclei in early degenerative phases. It is therefore reasonable to assume that this blue staining indicates a degenerative condition in the liver cells at a very early stage, since it appears before histologically degenerative changes can be demonstrated in the cell and is not found to any significant extent in cells which show obvious histologically degenerative changes. Nor have mitochondria changes, which seem to be an early degenerative sign in liver cells, been observed in these isolated blue-stained cells.

On increasing dosage, 10—12 mg. thyroxin, it is seen that more cell groups, and always groups which lie close to the dilated sinusoids, take the blue stain. In this case the preparation looks like a map. The same cells still show no signs of increased permeability for larger particles, *e. g.*, hydrocollag. Nor are there any other morphological signs of cell degeneration except mitochondria changes.

However, at these doses (10 mg.) there are certain changes in the finer histological structure of the cells, the mitochondria. These appear normally in Bensley or Altmann-stained sections as round, regularly large, lightly red-stained bodies which are present in large numbers in the cytoplasm of liver cells. However, in the cells which comprise other kinds of liver tissue, *e. g.*, the gall duct epithelium, they are very scarce. As these formations are regarded as related to the metabolic processes, this fact agrees well with the more intense metabolic activity of the liver cells. In the liver there is some normal variation in the mitochondria content of the cells, as the peripheral cells in the lobuli contain markedly more mitochondria than those which are more centrally situated (Fig. 8). However, after thyroxin doses of 8—10 mg., this difference in mitochondria content is eliminated. This is due to the fact that the central cells become more rich in mitochondria than in the normal controls, while the mito-

chondria content in the peripheral cells does not seem to decrease, judging from arbitrary observations. However, we have made no mitochondria counts. This observation seems to indicate that the central cells have undergone progressive changes, possibly an increased function of compensatory nature. When larger thyroxin doses are

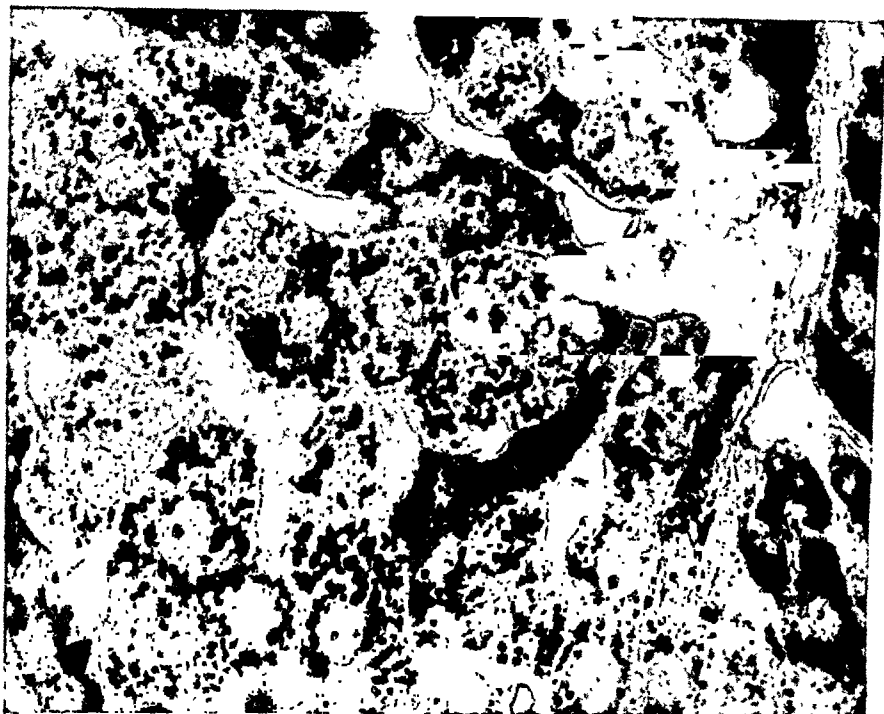


Fig. 8.

× 1000. Control rabbit. Mitochondria staining after Altmann. Abundant mitochondria in peripheral part of lobulus to the left.

administered there are marked regressive changes in the mitochondria. They become larger, their contours grow less sharp (Fig. 9) and, with the staining methods employed, the clear red color becomes more bluish. Within the actual mitochondria, regions may be seen which resemble vacuoles. Finally their number within the individual cells is reduced. In cells which show pronounced histological indications of degeneration they may disappear completely (Fig. 10). These mitochondria changes, which may be assumed to be the first histological indications of cell degeneration, occur however after the above-described vascular changes and permeability changes in the cells as regards colloidal vital dyes.

No signs of progressive changes in the liver cells, with the exception of the above described mitochondria changes, have been observed with certainty in this material. Here and there are found numerous binucleated liver cells but this has no relation to the

thyroxin dosage. No mitotic activity has been observed in the nuclei and the number of binucleated cells in a normal liver may be so high that a determination of normal and pathological values is difficult.

In doses over 14 mg. thyroxin there are pronounced histological

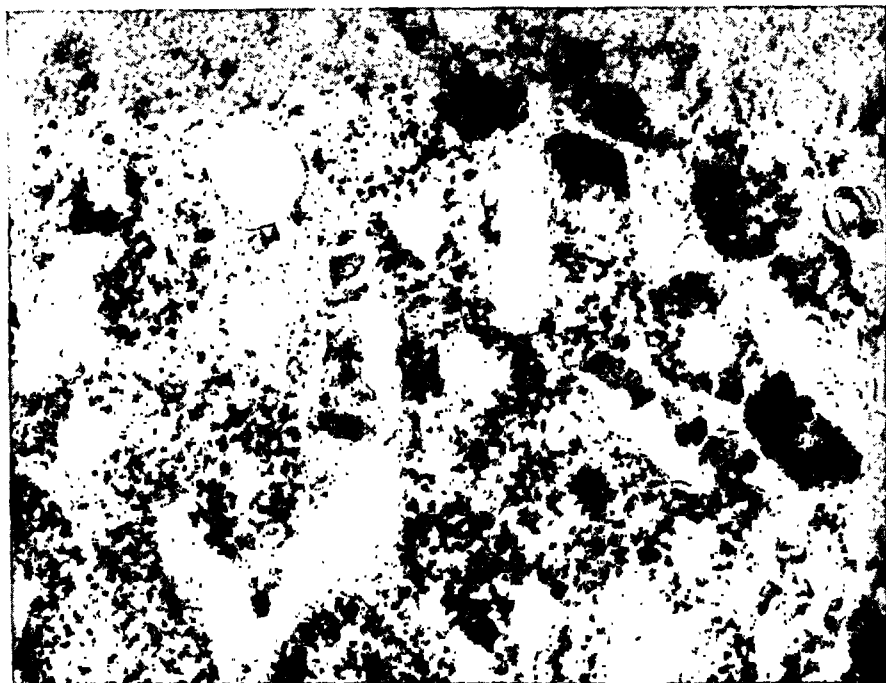


Fig. 9.

× 1000. Rabbit No. 9, thyroxin dose 14 mg., observation 8 days. Mitochondria staining after Altmann. Regressive changes in the mitochondria.

indications of regressive changes. The cells exhibit vacuolisation of the cytoplasm and sometimes also of the nucleus. The first sign is usually abnormal chromatin distribution within the nucleus. In the later development there may also be structural changes of a serious nature, even pycnosis and rheksis, or a total cell necrosis. Even in these cases of liver parenchyma or gall ducts may be found, but possibly in the reticular supporting tissue. On the whole, it must be said that the regressive changes dominate at these dosages. Liver cell atrophy, which has been discussed as a central and primary occurrence in the liver in experimental hyperthyrosis, in in our material a very late-occurring regressive phenomenon, which is moreover a general cytological rule. The degenerating liver cells often lie spread throughout the lobe with no particular localisation. However, it is frequently seen that they have a tendency to be abundant centrolobularly, just around the central vein. This is especially pro-

nounced in rabbits which die spontaneously, where large centrolobar areas show pronounced regressive cell changes.

The glycogen content of the cells shows marked reduction after doses of 6 mg. Judging from our material, the glycogen begins to disappear centrally in the lobuli. At doses of 6—8 mg. there are

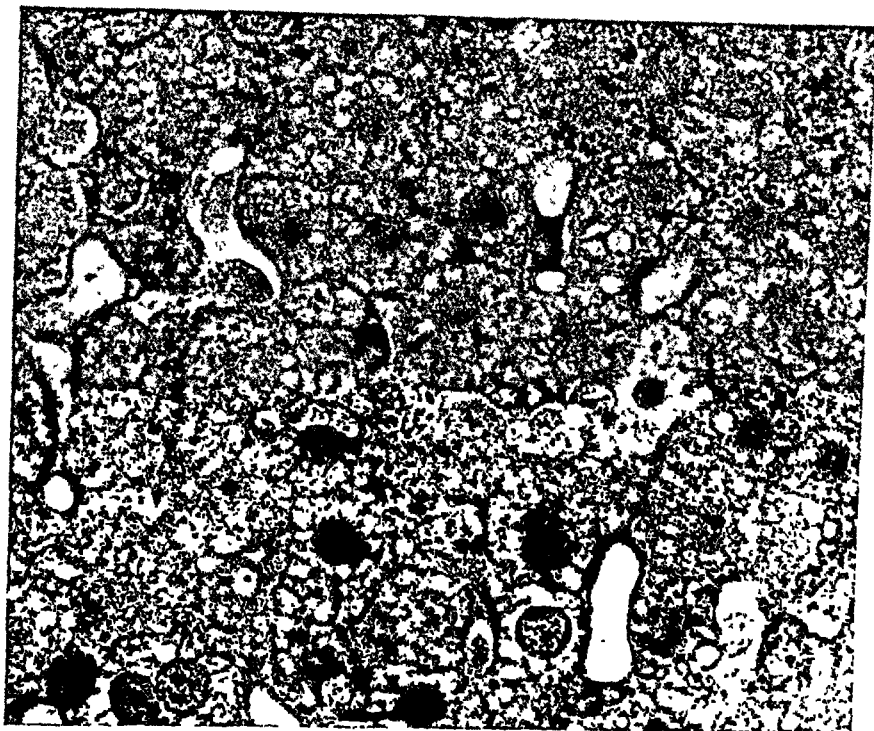


Fig. 10.

× 1000. Rabbit No. 13, thyroxin dose 20 mg., observation 10 days. Mitochondria staining after Altmann. Disappearance of mitochondria in degenerating cells.

usually only traces of glycogen granules peripherally toward the interlobular formations. Frequently the glycogen granules may have a peculiar half-moon localisation to one side of the cytoplasm. At higher doses of thyroxin the liver may be regarded as completely free of glycogen with the staining methods employed.

Examination of fat stained sections reveals no indication of increased fatty deposit in the liver cells, either during the early or later phases of liver cell degeneration. Abundant fat deposit is seen only in animals which have died spontaneously, often localised to the centrolobular cell- degenerated areas.

3. Changes in the supporting tissue. As these investigations were made with regard to early changes in the liver and the animals have a maximum observation time of only 14 days, it was not to be expected that significant changes would be found in the supporting tissue. And the collagenous supporting tissue shows no signs of re-

action. But the reticular tissue elements show moderate but definite changes at the higher doses. This is true first and particularly of the fibrils which lie directly in the walls of the sinusoids. After doses of 14—16 mg. they become thicker, somewhat branched and the contours are uneven. They seem to have a strong affinity for trypan

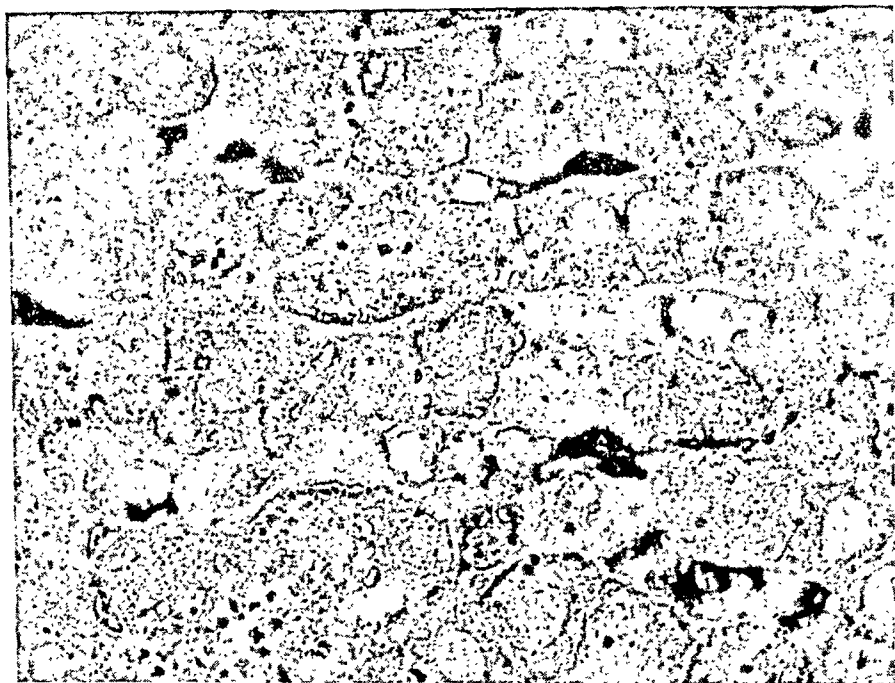


Fig. 11.

× 1000. Controle-Rabbit. Silver impregnation after Laidlaw.

blue and are therefore readily observable in ordinary sections from animals which have been injected with this dye in large quantities, and appear as deeply blue-stained formations. At the highest doses which we have employed they seem to be increased in number (Figs. 11 and 12) and the above-described changes should therefore probably be interpreted as incipient proliferative changes. These changes are most pronounced peripherally in the lobes. Whether these reticular fibrils can develop into regular collagenous fibres and thereby form the first stage of a liver sclerosis — two very important histopathological problems — cannot be determined from our material because of the short observation period. No increased local accumulation of histiocytes has been observed in our experiments during this proliferation process.

4. *Changes in the Kupffer cells.* After doses of 10—12 mg. thyroxin it seems that the functional potential of the Kupffer cells is increased. Their capacity to deposit trypan blue in granular form is

intensified. The same is true to a certain extent as regards deposition of hydrocollag. Thus a few minutes after intravenous injection of this substance it is seen that the particles are to a great extent bound to the surface of the Kupffer cells, while this does not take place until much later in normal controls. This increased

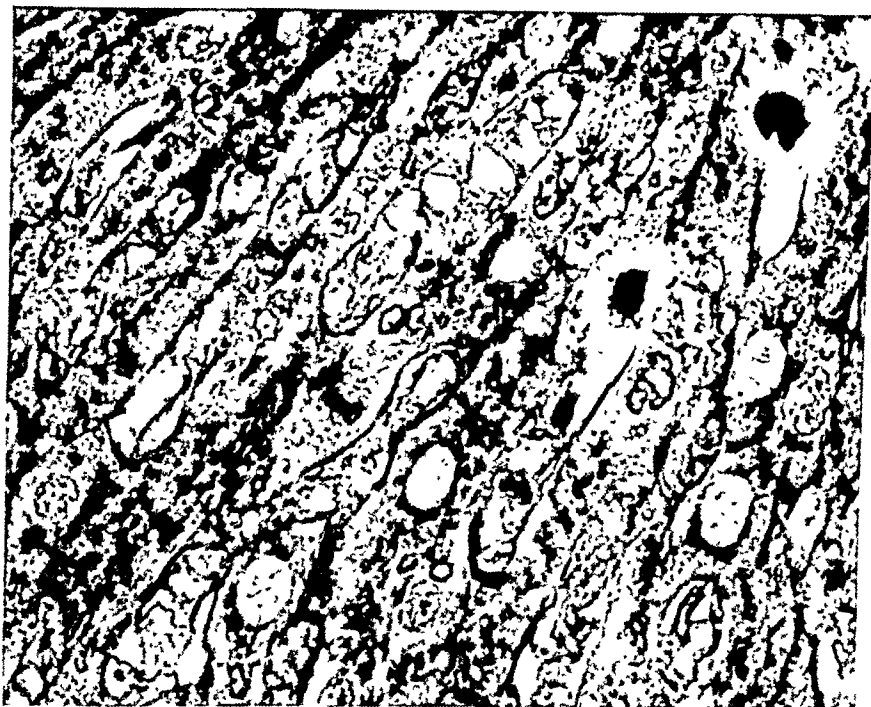


Fig. 12.

× 600. Rabbit No. 43, thyroxine dose 20 mg., observation 10 days. Silver impregnation after Laidlaw. Increased number of reticular fibrils.

activity also has a morphological effect, although slight. After the largest doses in this animal group it is found that the cells are somewhat larger than usual, more irregular in their contours and are more prominent in the vessel lumina. It also seems that they are more numerous. The nuclei also show changes in form and become more pronounced, more deeply stained and distinct. However, no deposit of cellular products has been definitely demonstrated in their cytoplasm. This increased activity of the Kupffer cells is always found in connection with all conditions where there is increased cell degeneration in the organism and thereby also increased tissue renovation. This phenomenon is generally found in connection with the reticulo-endothelial apparatus as a whole and is no peculiar phenomenon for the Kupffer cells. Activation of the Kupffer cells in hyperthyrotic conditions is thus not necessarily a direct consequence of thyroxine. The possibility must also be borne in mind that the

Kupffer cells deposit thyroxin as a detoxicating process, eventually together with their vehicle substance and this mechanism may be the direct cause of proliferation of the Kupffer cells.

Group II.

As mentioned above, this group consists of 5 animals which were given 1 mg. thyroxin daily in the course of 8 to 23 days. The doses were 7, 12, 16, 19, 22 mg. The histological and histochemical findings are on the whole analogous with those found for Group I, except that they are less pronounced for the same length of time, which is explained by the weaker thyroxin dosage. Vessel changes in the form of dilation and permeability are less pronounced even when only the dosage is considered. Thus, in this group, after a dose of 7 mg. the changes are much slighter than after 6 mg. in Group I. This is perhaps true particularly of the erythrocyte content of the dilated sinusoids which is strikingly less reduced than in the preceding group. The same is true of the liver cells. Mitochondria changes are observed only as a change in distribution in the form of increased mitochondria in the centrolobular liver cells, while the pronounced regressive changes in the mitochondria are not observed. The same is true of the general degenerative changes. Glycogen also disappears at much higher total doses in this group than in the previous one. Traces of glycogen are found at doses up to 13 mg. thyroxin. In the supporting tissue and Kupffer cells the changes are very slight but are principally the same as in the preceding group.

Group III.

This animal group consists of 4 rabbits which were given single doses of thyroxin varying from 3 to 8 mg. and with observation periods from 40 minutes to 5 days. The total doses were 4, 8, 11 and 15 mg. and the observation periods were 40 minutes, 75 minutes, 3 and 6 days, respectively. This group shows the same principal changes as Group I, but more accentuated. However, it should be mentioned that in one animal, which was injected with 4 mg. and was killed 40 minutes later, there was only slight capillary dilation and no other definite pathological findings. The glycogen content of the cells was normal. There were no permeability changes with trypan blue and no histological indications of progressive changes either in the liver cells, supporting tissue or reticulo-endothelial tissue. In one rabbit which was killed after 75 minutes, there was similarly moderate capillary dilation, none of the usual degenerative changes but there were signs of incipient permeability changes. The glycogen content seemed to be normal.

Group IV.

This consists of 7 animals which were given 1 mg. elityran daily and were killed in the course of 1—21 days at regular intervals. The doses were 6 cc. (2 animals), 8 cc., 10 cc., 17 cc., 21 cc. and 22 cc. elityran. For this group also the liver changes are principally the same as for the thyroxin-injected animals and they exhibit the same parallel increase of degree of organ findings on increased dosage. The first positive findings are observed after a dose of 3 cc. elityran. Here also the vascular changes appear first, somewhat earlier than the liver changes. Thus, capillary dilation can be demonstrated before there are definite intracellular changes in the form of mitochondria changes or glycogen reduction.

Group V.

This group consists of 10 rats which were injected with 1 cc. elityran daily from 9 to 40 days. The total doses were 9, 13, 19, 20, 22, 26, 29, 33 (2 animals) and 40 cc. elityran. In spite of the fact that this dosage was much higher in relation to the weight of the animals than for the rabbit group and that the observations were carried out over a longer period in some cases, the findings in the liver are much less pronounced in this group. After 4—5 cc. elityran the vessel and liver cell changes are barely detectable, and even after the largest doses, 20 cc., the vascular changes are very moderate. However, here also the vascular changes dominate both as primary and later findings. However, for this group there is no parallelism between increased dosage and the degree of the histological findings as was the case in the other experimental groups. The most extreme changes are observed after relatively small doses, 7—8 cc., and on higher dosage there is practically no more change. Thus a trace of glycogen may be detected in the liver cells even after the largest doses. This finding falls in line with observations on the general condition of the animals, as it is seen that they lose weight rather rapidly during the first few days and their general condition is somewhat weak, but after a time they begin to gain weight and some of the animals almost regain their initial weight at the end of the experiment. This may indicate a stronger resistance in rats against this substance than in rabbits. It is also possible that the animals may develop a kind of immunity against the substance. Metabolism controls would have been of interest here but have not been made on our material. With these reservations it can be said that the rat material demonstrates principally the same findings as the rabbit material.

Discussion.

In this material of experimental hyperthyrosis in rabbits and rats, all the experimental groups show evidence that the vascular changes are the dominating ones among the primary changes. Thus dilation of the liver sinusoids is seen after minimal thyroxin doses. These changes become more pronounced, one may almost say that they increase parallel with increased dosage. This capillary dilation is diffusely distributed throughout the liver, but as far as the individual lobulus is concerned it is markedly perilobular. This capillary dilation leads at an early stage to pronounced circulatory disturbances. This circulatory disturbance is expressed first as a probably increased permeability of the capillary wall for medium-sized colloid particles (trypan blue solution). Another and probably equally significant pathogenetic factor which characterizes this circulatory disturbance is the erythrocytopenia which is observed in the dilated sinusoids except in the animals which die spontaneously. This indicates that circulation in the sinusoids peripherally in the lobuli is abnormal. The same observation is made after somewhat larger thyroxin doses on injection of the corpuscular substance, hydrocollag, as this also leads to a relative blockage of the sinusoids peripherally in the lobuli. It is difficult to say anything definite as to the mechanism of this circulatory disturbance, but it must be taken into consideration that the circulation in the liver sinusoids peripherally in the lobes probably deviates from that in the other parts of the lobes under normal conditions, since branches of the arteria hepatica are said there to be confluent with the portal vein system.

At larger thyroxin dosage there is oedema in the capillary walls, and at the highest dosage employed, also pericapillary oedema and in rare cases degenerative changes with shedding of the vein epithelium. These early findings, which have not been systematically investigated previously, agree well with the results of a number of other investigators who have also emphasized the vascular changes, as the most significant in the pathogenesis of liver changes in experimental hyperthyroidism (Haban, 1935; Henlein & Dickhoff, 1936; Sciaky, 1938). Investigators who have examined spontaneously dead animals have described blood congestion in the sinusoids (Gerlei, 1932; Zeldenrust & van Beek, 1939). We have made the same observation in spontaneously dead animals but are inclined to regard it as an agonal phenomenon.

The usual cytological signs of degeneration of the liver cells are insignificant in comparison to the vascular changes at the thyroxin doses we have employed. After the largest doses in our material we have demonstrated only moderate degenerative changes. Larger areas of necrotic liver cells which have been described after large thyroxin doses (Gerlei, Haban et al.) have not been observed in our material.

Only irregularly and diffusely have we found individual liver cells in transition to necrosis. However, in animals injected with larger quantities of trypan blue solution after small thyroxin doses, as an early change we have observed diffuse blue staining of the cytoplasm and nucleus in isolated liver cells. These cells have had a constant localisation to the walls of the sinusoids which are most dilated, and have thus been located peripherally or intermedially in the lobuli. This blue staining is interpreted as a permeability increase for relatively small colloids in the anatomical complex: vascular wall liver cell membrane at the same point. The determination of which of these two components is the object of this pathological increase in permeability is difficult, as the normal histology of the liver is not completely investigated in this field. Judging from our experiments, it seems that the liver cell membrane is constantly pathologically changed in the blue-stained cells.

The functional-histological change in these liver cells should not categorically be interpreted as being of a regressive nature. However, it is found that not only the cytoplasm, but also the nucleus is stained blue, which should indicate that the nuclear membrane also exhibits the same permeability disturbance. We know that in normal cells the nucleus practically never takes vital stain, while this does happen in cells in the degenerative phase. In spite of the fact that no other cytological indications of degeneration can be demonstrated in these cells, it still seems that this is an indication that these cells are in a regressive phase. It cannot be stated whether this condition is of a reversible nature. However, the normal mitochondria situation in these cells indicates that the finer cell structures are intact so that restitution should be possible. The difficulty in solving this problem is even greater because the blue staining, interpreted as a degenerative cell process, only represents a very limited period of time within the degenerative phase of the cell. Cells with marked degenerative changes or on the border line of necrosis do not show this blue staining to any great extent.

The investigators of experimental hyperthyrosis who regard the vessel changes as the most important formal genetic factors of liver changes, have ascribed most significance to the pericapillary oedema. However, as discussed above, our investigations have shown that in experimental hyperthyrosis a number of other changes in circulation arise earlier than the pericapillary oedema. And judging by other investigations this seems to have been observed only after large doses. And even then they do not seem to have been found extensively in the liver. In our experiments pericapillary oedema only appeared locally after doses of 20—22 mg. thyroxin. But at an earlier stage we have demonstrated changes in the vascular walls which we have designated as capillary oedema. This has been found almost constantly in the sinusoids where the circulation was poor. It is reason-

able to assume that this capillary oedema, but is in a way an earlier stage of the latter, so that the difference is only one of degree, in other words due to a combination of dosage and time element. Our investigations lend no evidence to a denial of the occurrence of pericapillary oedema as some investigators have done (Heinemann, 1937; Zeldenrust & van Beek, 1939) or to regard it as uncertain (Strøm, 1942). On the basis of our animal material we are inclined to assume that pericapillary oedema has been too strongly emphasized as an early finding in the formal genesis of the hyperthyrosis liver and are inclined to lay more weight on the capillary oedema, as this occurs earlier and is more diffusely distributed and more pronounced than the pericapillary oedema. According to our material, pericapillary oedema represents a more advanced and more serious circulatory disturbance as even in moribund or spontaneously dead animals it has not attained any diffuse development in the liver.

We regard the following sequence as significant for the formal genesis of the liver changes as observed in our material:

Circulatory disturbances in the sinusoids with erythrocytopenia → permeability disturbances for small colloidal stains in the anatomical complex capillary wall — liver cell membrane → capillary oedema (more rarely pericapillary oedema) → the usual histological regressive liver cell changes.

It seems natural to assume that there is a definite relation between these observations so that the primary capillary dilation with erythrocytopenia leads to an hypoxia in the tissue with resultant parenchymal injuries. It must, of course, be borne in mind that in addition there may be a direct toxic effect on the liver cells. Most investigators of experimental hyperthyroidism describe dilation of the liver sinusoids. However, this observation is variously evaluated. Some investigators (Gerlei; Zeldenrust & van Beek) emphasize the significance of cardiac congestion as a causal factor but their observations were made on spontaneously dead animals which often show signs of cardiac congestion while this is not found in animals which are killed. Schönholzer and others interpret capillary dilation as a passive result of the liver cell atrophy. We disagree with this interpretation because the capillary dilatation may be observed at a time long before there is any atrophy or degeneration of the liver cells. The objection may be raised that according to many anatomists the liver sinusoids lack Rouget cells and thus also lack the ability of active changes in the lumina. However, it has been found that lymph vessels, which also lack Rouget cells, may undergo active dimensional changes. Moreover it has been demonstrated by experiments with sympaticus- and parasympaticus-effective substances that the liver sinusoids may become dilated without any quantitative or qualitative changes in the liver cells. Liver cell atrophy has, on the

whole, only been observed sporadically after the largest doses in our material. Moreover, as a general cytological degenerative phenomenon it is no early symptom. There seems therefore to be no basis for regarding liver cell atrophy as the primary finding in the hyperthyrosis liver.

The slight changes in the supporting tissue, which we have observed as proliferation of reticular fibrils peripherally in the lobe, cannot be ascribed much significance in the development of chronic sclerosis of the liver, even though such changes fit well into the picture. Our material affords no basis for the evaluation of the mechanism of this fibril formation which is a generally much contended histological problem. That it is an acellular process as assumed by Rössle is a question which cannot be solved by examination of sections of human or animal material but would be better adapted for in vitro experiments. It is also of subordinate significance for the problems treated in the present investigations.

Conclusions.

1. In an animal material which consists of 30 rabbits and 10 rats the early appearing liver changes in experimental hyperthyrosis have been investigated.
2. The earliest and most dominant are vessel changes in the form of dilation of the sinusoids with erythrocytopenia, permeability changes and capillary oedema.
3. Ordinary histologically demonstrable liver cell changes appear relatively late and then as degenerative changes.
4. A relatively early change in the liver cells is an increased permeability of the liver cell membrane and the nucleus membrane for colloidal vital dye (trypan blue). This condition is interpreted as a regressive cell process of functional nature.
5. Atrophy is a late occurring change in the liver cells and thus cannot be regarded as a primary change or as a significant cause of the dilation of the liver sinusoids.
6. The liver cell changes have their natural explanation in the tissue hypoxia which may be assumed to arise as a result of the demonstrated circulatory changes. Whether there is in addition a direct toxic effect of thyroxin on the liver parenchyma cannot be excluded.
7. Mitochondria studies seem to indicate that the liver cells, at the beginning of the thyroxin effect, are mobilised functionally in all parts of the lobe. This is possibly a compensatory phenomenon.
8. In the supporting tissue the collagenous tissue shows no reaction to the thyroxin doses employed. The reticular tissue shows moderate proliferation.
9. The Kupffer cells show functional, and to a lesser degree, anatomical signs of activation.

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INCREASED INCIDENCE OF TUMORS IN MICE AFTER INTRAVENOUS INJECTION OF 9:10-DIMETHYL-1:2-BENZANTHRACENE

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(Received for publication December 30th, 1943).

The experiments to be presented here were carried out for further study of the effect of carcinogenic hydrocarbons injected intravenously.

Previously this method of application has been employed only in relatively few experiments.¹⁾ It is of considerable interest, however, because experiments on inhibition of the growth of malignant tumors by means of carcinogenic hydrocarbons have shown that a sustained effect from the administration of these substances may be expected only when they are injected intravenously. Moreover, it is found that with this method of application the treated animals (mice) are able to tolerate even very large doses of the substances — doses which would kill the animals within a few days if injected subcutaneously or intraperitoneally (Stamer, 1943).

The present experiments were carried out with a suspension of 9:10-dimethyl-1:2-benzanthracene in water in a concentration of 1 ‰.²⁾

The animals employed in these experiments were mice of strain Little's dilute brown (Dlb), the Furth strain Aka, strain Black and strain Street — *i. e.*, animals with widely different disposition to tumor formation. In strain Dlb nearly 50 % of the females over 9 months have tumors (mammary carcinoma), and about 2 % of males as well as females over 8 months have leukemia. In Furth's strain Aka 50—70 % of the males and females over 8 months die of leukemia. Strain Black is practically »tumor-free«, only 1 ‰ of these mice dying of leukemia. In strain Street 25—30 % of the females

¹⁾These experiments were performed chiefly by Andervont (1939), Andervont & Lorenz (1937), Shimkin (1939) and Peacock & Beck (1938). The experiments are cited and discussed in a previous work by Stamer (1943).

²⁾As to the preparation of the watery suspension, see a previous work by Stamer (1943).

die of mammary carcinoma, and 1 % of males as well as females die of leukemia.

In order in each experiment to have the control material as exact as possible, the groups of experimental animals and controls were made up of »half litters«, *i. e.*, of each litter one-half of the young were employed as experimental animals, one-half as controls. As the strains here employed, excepting strain Street, are pure inbred strains, the animals are beforehand very much alike. The employment of »half litters« means an additional guarantee that the experimental animals and the controls are alike as to their disposition to tumor formation.

At the commencement of the experiment the animals were between two and five months old.

Experiment with Strain Dlb.

39 female mice of strain Dlb were divided into half litters. The experimental animals were given intravenous injections of 9 : 10-dimethyl-1 : 2-benzanthracene suspended in water, once a week for four weeks, altogether 2 mg. of the substance. When the first tumors

Fig. 1.

Lifetime of the individual animals reckoned from the day of the first injection and the cause of their death, in experiment with strain Dlb.

	10	11	12	13	14	15	16	Mths. after 1' inj.
Intercurrent diseases	● ● ●	●		● ● ● ●	●			
Leukemia				○ ●	●	○ ○	○ ○	
Leukemia + mammary cancer	○			●			○	
Mammary cancer		●			●			
		○	○ ○					

● Experimental animals.

○ Controls.

appeared, several animals had died of intercurrent disease. At this juncture (10 months after the first injection) 14 experimental animals were living and 10 controls.

Fig. 1 gives a schematic survey of the lifetime of these animals and the cause of their death.

From Fig. 1 it will be noticed that the experimental animals differed in no respect from the controls. Tumors were equally frequent in the two groups, and there was no acceleration of the tumors in the treated animals. The only finding that might suggest any effect of the carcinogenic hydrocarbon is the concurrent development of leukemia and mammary carcinoma in 1 treated animal.

Experiment with Strain Aka.

116 mice of Strain Aka were divided in half litters and in such a way that either half of the litter contained nearly the same number of males and females. The experimental animals were given intravenous injections of 9 : 10-dimethyl-1 : 2-benzanthracene in water once a week for four weeks, receiving thus a total dose of 2 mg. of the substance.

Fig. 2.

Lifetime of the individual animals reckoned from the day of the first injection and the cause of the death, in experiment with strain Aka.

	4	5	6	7	8	9	10	11	12	13	14	15	16	Mths. after 1 st inj.
Intercurrent diseases							● ● ●	●	● ● ● ●	●	●		●	
Leukemia	● ●	●	●	● ● ● ●	● ● ● ●	● ● ●	● ● ●	● ●	● ●		● ●			
		○ ○	○	○ ○ ○	○ ○ ○ ○	○ ○ ○ ○	○ ○ ○ ○	○ ○ ○ ○	○ ○ ○ ○	○ ○				

● Experimental animals.

○ Controls.

At the time when leukemia first appeared, several of the animals had died of intercurrent disease, so at this juncture (4 months after the first injection) 39 experimental animals were living, and 47 controls.

Fig. 2 gives a schematic survey of the lifetime of these animals and the cause of death.

From Fig. 2 it will be noticed that of the 39 treated animals 26 (*i. e.*, a little over 69 %) had leukemia, while of the 47 controls 30 (*i. e.*, nearly 64 %) had leukemia. This means that leukemia developed with practically the same frequency in the treated animals and in the controls. Further, there was no acceleration of the development of leukemia in the treated animals.

So in this mouse strain, too, the carcinogenic hydrocarbon had no particular effect on the tumor formation.

Experiment with Strain Black.

115 mice of strain Black were divided into half litters in such a way that the two halves included nearly the same numbers of males and females. The experimental animals were given intravenous injections of 9 : 10-dimethyl-1 : 2-benzanthracene suspended in water, once a week for four weeks, receiving a total dose of 2 mg. of the substance. When leukemia appeared in the first animal, several had died of intercurrent disease, and at this juncture (5 months after

Fig. 3.

Lifetime of the individual animals reckoned from the day of the first injection and the cause of death, in experiment with strain Black.

	5	6	7	8	9	10	11	12	13	14	Mths. after 1 st inj.
Intercurrent diseases				●	●	● ● ●	● ●	●	●	25 ●	killed
		○		○ ○			○ ○	○ ○		42 ○	,
Leukemia	● ●	● ●	●	●		●	● ●			●	,
Cancer of ovary										● ●	,

● Experimental animals.

○ Controls.

the first injection) 51 treated animals were living, and 49 controls. 14 months after the first injection the still surviving animals were killed (lack of food) namely, 28 experimental animals and 42 controls.

Fig. 3 shows the lifetime of these animals and the cause of death.

Fig. 3 shows that of the 51 experimental animals 11 had leukemia and 2 ovarian cancer, while — as expected — none of the controls showed any development of tumor. Thus there was an enormous increase in the incidence of leukemia, as nearly 22 % of the treated animals had leukemia as against 1 ‰ under ordinary conditions, and in addition 2 of the treated animals had cancer of the ovary.

Experiment with Strain Street.

108 females of strain Street were divided into half litters. The experimental animals were given intravenous injections of 9 : 10-dimethyl-1 : 2-benzanthracene suspended in water, once a week for 4 weeks, receiving thus a total of 2 mg. of the substance. When the first tumor appeared, several of the animals had died of intercurrent disease, so at this juncture (4 months after the first injection) 51 experimental animals were living, and 47 controls.

Fig. 4 shows the lifetime of these animals and the cause of death.

Fig. 4 shows a considerable difference between the experimental animals and the controls.

Of the experimental animals 16 (31 %) died of leukemia as against 3 (a little over 6 %) of the controls. Of these 16 treated animals 1 had both leukemia and cancer of the lung; of the 3 controls 1 had both leukemia and adenoma of the lungs.

Mammary carcinoma appeared in 4 experimental animals, 1 of which had also cancer of the lung; of the controls only 1 had mammary carcinoma. Cancer of the lung was found in 5 experimental animals. In addition, 3 experimental animals had pulmonary adenoma, suspect of carcinoma. In contrast to this, none of the controls had cancer of the lung, whereas 3 controls had pulmonary adenoma. Finally 1 experimental animal had cancer of the ovary.

Thus there can be no doubt that 9 : 10-dimethyl-1 : 2-benzanthracene has caused a great increase in the incidence of leukemia, besides bringing about that cancer of the lung appeared in 5 experimental animals. As to mammary carcinoma and cancer of the ovary, the figures are too small to allow of any definite statement.

Conclusion.

The interpretation of these experiments is rather difficult. Two of the strains of mice employed responded to the treatment with a greatly increased incidence of tumor formation, while two strains showed no reaction to the treatment.

	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	Mths. after 1' inj.
Intercurrent diseases	• •	•	•	•		•	•	•	•		•	• • •	•	•	• • •	•			• •					
	○ ○ ○	○		○ ○ ○ ○	○				○ ○ ○	○		○			○ ○ ○	○ ○ ○	○ ○ ○	○ ○	○	○	○ ○ ○	○ ○	○ ○ ○ ○ ○ ○ ○	
Leukemia	•	•		•	•	•	•				•		•	•	•	•			•					
Leukemia + Cancer of lung									○	○									•					
Leukemia + Adenoma of lung																								
Mammary cancer													• •	•								○		
Mammary cancer + cancer of lung						○												•						
Cancer of ovary							•																	
Cancer of lung									•									• •						
Adenoma of lung suspected of cancer																•		•						
Adenoma of lung											•													
																			○				○	

In strain Aka, in which leukemia is a very frequent cause of death, the frequency of tumor formation was not influenced by the given treatment, whereas in strain Black, in which leukemia ordinarily is a very rare phenomenon, the same treatment resulted in a very marked increase in the incidence of leukemia, and a few of these animals had also cancer of the ovary.

As to strains Street and Dlb, in which leukemia is a rather rare cause of death and mammary carcinoma a frequent cause of death, one strain (Street) showed a great increase in the incidence of leukemia in response to the treatment and, in addition, several of the treated animals had cancer of the lung, and one animal had cancer of the ovary, whereas there was no distinct increase in the incidence of mammary carcinoma. In contrast hereto, the other strain (Dlb) showed no increase in the incidence or acceleration of leukemia or mammary carcinoma.

Engelbreth-Holm & Lefevre (1941) have treated mice of strains Aka and Dlb with 9 : 10-dimethyl-1 : 2-benzanthracene by application to the skin and by subcutaneous injection and found, besides development of local tumors at the site of application, an acceleration and increase in the incidence of the tumors characteristic of these two strains — that is, leukemia in the case of strain Aka, mammary carcinoma and leukemia in Dlb. In an experiment with strain Dlb the mice were given 0.5—1 mg. of 9 : 10-dimethyl-1 : 2-benzanthracene subcutaneously; of 19 treated animals 4 had leukemia, 3 mammary carcinoma, 1 lymphosarcoma and 1 ovarian cancer, while none of the controls showed any development of tumor, within the age of 8 months. Here, then, there was a very marked increase in the incidence and acceleration of the tumor growth, and this result forms a striking contrast to the outcome of my experiment with strain Dlb, in which none of the 14 treated animals presented any tumor development prior to the age of 10 months, and tumor formation was not more frequent in the treated animals than in the controls. In an experiment with mice of strain Aka, Engelbreth-Holm & Lefevre injected 1 mg. of 9 : 10-dimethyl-1 : 2-benzanthracene subcutaneously; of 12 treated animals 9 had leukemia before the age of 8 months, while none of the controls had this disease. In my experiment with strain Aka none of the treated animals had leukemia before the age of 8 months, and the occurrence of leukemia was not more frequent in this group than in the controls.

Engelbreth-Holm & Lefevre obtained practically the same results with the two strains when 9 : 10-dimethyl-1 : 2-benzanthracene was applied to the skin of the back.

So it seems safe to say that the reaction of these two strains of mice to treatment with 9 : 10-dimethyl-1 : 2-benzanthracene depends on the method of application, as subcutaneous injection of, and painting with, this substance gives an increase in the incidence

of the malignant tumors characteristic of the two strains of animals, whereas intravenous injection of the substance gives no increased tumor incidence.

As to strains Black and Street, no experiments have yet been reported in which the animals were treated with subcutaneous injection of the substance or with its application to the skin. So nothing may be said as to whether these strains too will differ in their reactions to the substance, depending on its application. But it can be established that in contrast to strains Aka and Dlb, these two strains respond to intravenous injection of 9 : 10-dimethyl-1 : 2-benzanthracene with a marked increase in the tumor incidence.

Summary.

Mice of strains Dlb, Aka, Black and Street were given an intravenous injection of 9 : 10-dimethyl-1 : 2-benzanthracene suspended in water, once a week for 4 weeks, receiving a total dose of 2 mg. of the substance.

Strains Dlb and Aka showed no difference in the incidence of tumors in the treated animals and in the controls. In strain Black, on the other hand, the tumor incidence was enormously increased in the treated animals, as about 21 % of them had leukemia, whereas none of the controls had leukemia. In strain Street, too, the tumor incidence was greatly increased in the treated animals, as about 31 % of them had leukemia as against 6 % of the controls; further, several of the treated animals had cancer of the lungs, while there was no sure increase in the incidence of mammary carcinoma.

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STUDIES ON THE COMPENSATORY HYPERTROPHY OF THE FETAL ADRENAL GLANDS IN THE ALBINO RAT, PRODUCED BY ADRENALECTOMY DURING PREGNANCY

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(Received for publication January 12th, 1944).

During pregnancy there is assumed to exist a functional correlation between the endocrine organs in the fetus and the maternal organism. As regards the adrenal cortex experimental biological investigations during gestation have yielded proofs of such correlation with functional activity in the fetal adrenal glands.

Firor and Grollmann (1933) have investigated the problem by performing adrenalectomy in albino rats during pregnancy. They found that the period of survival of the animals after the operation was increased to the double in comparison with non-pregnant animals, or on the average from 7 to 15 days. Stewart (1912) attained the same result in cats, and Stewart and Rogoff (1927) in dogs.

Billmann and Engel (1939) have demonstrated this phenomenon in a dog, which was adrenalectomized in the later stage of pregnancy. After the animal had been brought through the crisis immediately succeeding the operation by artificial administration of cortical hormone, the pregnancy proceeded normally without pronounced symptoms of adrenal insufficiency. Directly after the birth of 6 living pups, however, the animal passed into a state of acute adrenal insufficiency, with fatal issue after the lapse of 36 hours. On autopsy no accessory suprarenals were found in the mother, and the fetal adrenal glands must be assumed to have exercised the vicarious function, together with the endocrine function of the placenta.

Biological investigations made by Ingle and Fisher (1938) point in the same direction: After adrenalectomy of albino rats during pregnancy they observed an increase in weight of the fetal adrenals at birth, as compared with normal control animals. The same was found by Finn Boe (1937—unpublished work).*)

In the present work we have sought by *histological* investigations to obtain information as to the functional state of the fetal adrenal glands. The work embraces an examination of the cytology of the adrenal glands,

*) Personally communicated.

as well as a registration of their mitotic activity. The investigations were carried out on albino rats and the material comprises two groups:

- 1) Normal fetal adrenals examined at term.
- 2) Corresponding fetal adrenals in cases where the mother was adrenalectomized in the later stage of pregnancy.

From earlier investigations it must be assumed that hyperfunction of the fetal adrenal glands sets in after such adrenalectomy. In our investigations we have used the mitotic activity of these glands as a histological test for the functional state. Augmented mitotic activity shows that increased proliferation is taking place, and points in the direction of functional hyperactivity.

Through our investigations of the adrenal cytology we have sought to obtain a histological picture of the organ's secretory activity. This problem has received little attention as regards the adrenal glands, and especially little as regards the fetal adrenals. Goormaghtigh (1922) was the first to consider the cytological structure of the adrenal glands in direct relation to their secretory activity. By staining with ironhematoxylin he found the so-called »siderophile« cell inclusions. He assumed that these represented an active secretory stage in the cell, associated with the internal secretion of the adrenal glands. Hoerr (1936), on the contrary, regards the »siderophile« granules as a sign of incipient cellular degeneration and denies their significance as secretory granules. Broster and Vines (1933) have by a quite different method of staining, with acid-fuchsin, found a »fuchsinophile« cell type and supposes this to be associated with secretory activity with special relation to androgen output of the adrenals. O. Torgersen (1940) has demonstrated seasonal variations in the light cell types of the adrenal cortex, with relation to the sex hormones.

In connection with our investigations of the cytological structure of the adrenal glands we shall have occasion to discuss these problems in general.

General experimental conditions and technique.

For the investigations were employed albino rats of one and the same strain. The rats were kept under optimal conditions, with a constant temperature of 21° C. (thermostat regulation) in the room, which was ventilated by means of an electrical fan arrangement, and they were given an all-round standard diet. For the experiments were used animals 3 or 4 months old, weighing from 180 to 220 g. at the time of insemination, which was effected by placing females in sexual phase together with potent males. The exact time of conception was thus registered.

In the adrenalectomized group extirpation of the adrenal glands was carried out on the 16th day of gestation, *i. e.*, at a time when the fetal adrenals already appear as a distinctly oval heap of cells. The technique employed for the extirpation was in accordance with Firor & Grollmann's suggestions (1933). The operation was performed in ether narcosis, lasting about 10 minutes. The adrenal glands were removed by means of a lumbar incision, and they were dissected out without squeezing or damaging the organ. As much as possible of the surrounding fat and connective tissue was removed at the same time. No considerable hemorrhage was recorded, and the animals

quickly recovered from the effects of the operation. Neither have any complications been noted in the days immediately after the operation.

Autopsy of all the animals was made at term, after $21\frac{1}{2}$ days of pregnancy, either immediately post partum or, where birth had not yet taken place, by Caesarian section. Thus the autopsy was made $5\frac{1}{2}$ days after extirpation of the glands in the adrenalectomized group, and the animals had in that time shown only slight signs of adrenal insufficiency.

Their weight remained unchanged or fell somewhat on the day after the operation, but afterwards increased considerably and was at the term only slightly under the weights recorded for the normal control animals. All the young were alive at birth and were well developed, being of about normal size (Table 1).

Weights of the fetal adrenal glands.

For comparison with previous investigations (Ingle and Fisher) we carried out weighing of the fetal adrenal glands in our animals. The technique of weighing was carefully standardized, and all weighings were done by one of the authors. The glands were rapidly dissected out, quickly transferred to a closed glass tube and all adrenals from the fetuses of each mother were weighed together. The average weight per pair of fetal adrenals for each pregnant rat was thereupon calculated.

The results are as follows (Table 1): The adrenal glands in the control group have an average weight of 2.61 mg. per pair, with a standard deviation of ± 0.25 . The fetal adrenals of the adrenalectomized group have an average weight of 3.47 mg. per pair, with a standard deviation of ± 0.38 . This represents an increase in weight of 33 per cent for the latter group.

Table 1.
Weights of the fetal adrenal glands.

	Number of animals	Number of fetuses per animal	Body-weight of fetuses g	Weight per pair of adrenals. mg.	Ratio: Wt. of gland mg. Body-weight g
Mother adrenalectomized 16th day of gestation	15	10 (3-14)	4.1 (3.5-5.1)	3.47 ± 0.38 (2.89-4.26)	0.85 (0.65-1.11)
Normal control animals	14	8 (3-13)	4.4 (3.1-6.4)	2.61 ± 0.25 (2.13-3.08)	0.62 (0.39-0.85)

After adrenalectomy in the later stage of pregnancy we have been able to note a distinct increase in size of the fetal glands at term. As already pointed out, this increase in weight may reasonably be regarded as a compensatory process, so that one might expect to find, parallel with the rise in weight, a more active functioning of the fetal adrenals. As will later be shown, this has been confirmed by our histological investigations.

Histological investigations.

Histological technique.

After being weighed, the adrenal glands are transferred as quickly as possible to the fixation solution. Flemmings fixation fluid has been employed, with very good results, the small organs being rapidly penetrated by the liquid. For the purpose of control some adrenal glands were fixed directly without previous weighing. The organs were left in the fixative fluid for 24 hours and then transferred to paraffin after dehydration. For such small organs this can be effected very rapidly, in the course of 24 hours. Other fixative solutions tried were Zenkers, Müller-Formol and formalin, but none of these yielded so good a picture of the cellular structure. Staining was affected partly with Haidenhain's ironhematoxylin, partly with Altmann's acid-fuchsin, both of which gave good results. Especially the »siderophile« and »fuchsinophile« cell types are brought out very distinctly with the technique employed, while the mitoses are likewise well preserved.

Development of the adrenal glands in fetal life.

For interpretation of the histological findings it is of importance to have a knowledge of the development of the organ. Pankratz (1931) detected the first signs of the adrenal cortex anlage in the rat on the 13th day of fetal life in the form of a thickening of the coelomic epithelium directly medial to the cephalic part of the mesonephros. Proliferation of the mesenchymal elements progresses rapidly and on the 16th day the adrenal cortex anlage forms an oval mass projecting into the coelomic cavity.

On the 16th day begins likewise the migration of the sympathetic cell elements into the cortical anlage and this migration continues right up to the moment of birth. These cells, which are later differentiated into the chromaffin cells and build up the adrenal medulla, come from the sympathetic ganglia dorsal to the adrenal cortex anlage. Pankratz has observed two different types of cells in the migrating cell masses. The majority of them are small dark-coloured cells, partly arranged in rosettes. Besides there are found larger and paler cells with vesicular nucleus. Ganglion cells are very seldom seen. From the 17th day nerve fibres are seen to migrate into the cortical anlage and these are in connection with the sympathetic anlage. At birth he finds the sympathetic cell elements in and around the vascular spaces in the central parts of the adrenal gland.

Histological structure of the adrenal glands.

a. The normal group.

The normal adrenal gland in the rat is at birth an oval organ surrounded by a thin capsule of connective tissue (Fig. 1). The zona

glomerulosa appears somewhat indistinctly with cells in three rows inside the capsule. The cells are small, of round or oval shape, and the nuclei are often rather dense. The cytoplasm stains with medium intensity and the cell boundaries are indistinct. The cells are quite

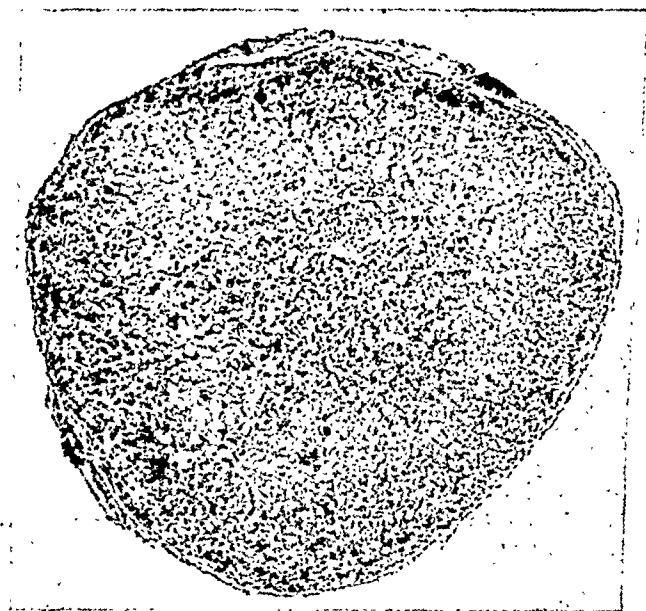


Fig. 1.

Normal fetal adrenal gland. Light-coloured cells in glomerulosa and fasciculata. Dark cells in a peripheral sector left and in central zone.

Haidehain's ferrohematoxylin. $\times 60$.

irregularly arranged and the typical glomerular arrangement is not yet to be seen.

The zona fasciculata, inside from the glomerulosa, forms the greater part of the organ, but likewise this portion has not yet acquired its typical character. The cells are not arranged in rows separated by radial blood vessels, such as is seen in the fully developed gland. The blood vessels are still few in number and not very prominent, especially in the outer third part of the organ. The cells lie close to each other and form solid masses. They are here larger than the glomerulosa cells. Most of them large, globular or rounded off in polygonal form, with a light-staining cytoplasm and a large round or oval, light coloured nucleus, poor in chromatin. The largest cells are found in the outermost part of the zone, and the cell boundaries are distinctly marked.

In accordance with the stage of development of the organ there is to be found no well-defined medullar zone. The central portion presents a very irregular picture with a mixture of blood sinuses, cortical cells in trabecular arrangement, as well as medullary ele-

ments. This central zone comprises about one-third of the diameter of the whole organ, measured on cross-section through the centre.

As is seen from the above description, the adrenal gland in the new-born rat has not yet attained the typical topography of the

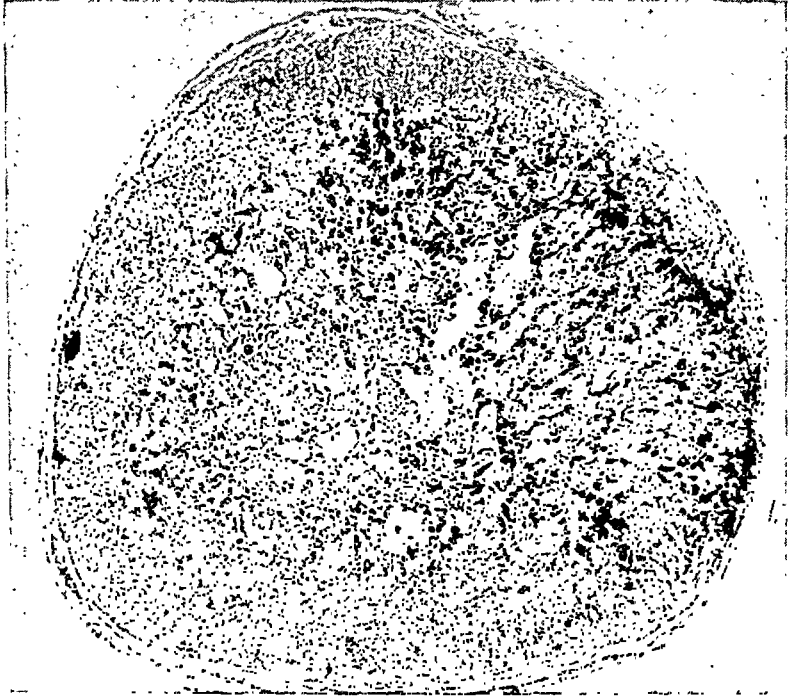


Fig. 2.

Fetal adrenal gland after adrenalectomy. Great hypertrophy of central zone.
Distended blood sinuses surrounded by darkgranulated cells.
Haidenhain's ferrohematoxylin. $\times 60$.

adult animal, and the division into the characteristic zones is not much in evidence.

As to the cytology of the gland the most distinctive feature is the occurrence of two different and highly characteristic types of cortical cells. The main body of the organ is built up of light-coloured, rounded, regular-shaped cells, such as described in the fasciculata. In strong contrast to these are seen cells of quite another type. They are of a smaller size, polygonal in shape with pointed offshoots and often with excavated contours. On staining with iron-hematoxylin they are dark in colour; and when stained with acid-fuchsin they are intensely red. They occur separately or as small collections of cells scattered amongst the light-coloured cells. At some places they are confluent and form large star-shaped figures without any distinct boundary between the individual cells (Figure 4). With their dark colouring and peculiar configuration they stand out very clearly

against the surrounding masses of light-coloured cells. The typical colour of the cytoplasm is due partly to a dark-coloured basal substance, partly to the cytoplasm's content of granules. These granules become black when stained with iron-hematoxylin and bright red on being stained with acid -fuchsin. Sometimes there are also found

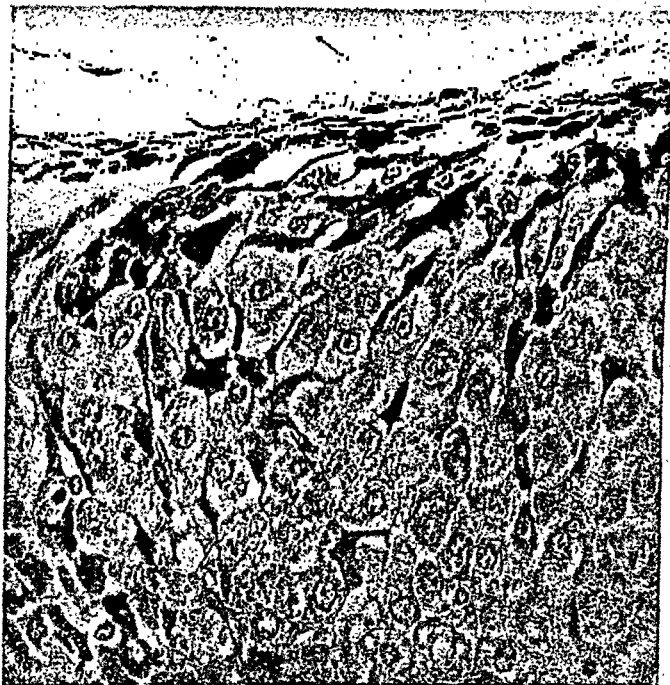


Fig. 3.

Dark-granulated cells in the glomerulosa in contact with the stroma cells of the capsule. Normal adrenal gland. Haidenhain's ferrohematoxylin. $\times 400$.

osmophile granules, which contribute to production of the dark colour. Pigment has not been detected in these cells, nor in other cells in our material. The nuclei of these cells are smaller than in the light-coloured cells, especially in the zona glomerulosa and in the outer part of the fasciculata. The nuclei are here often rich in chromatin and darkly coloured. The cells in their entirety then stain so darkly that it may be difficult to distinguish the individual cell structures.

These cells are found in all three zones of the adrenal anlage. In form and size they vary somewhat in the different zones, but they everywhere represent the same type of cell. In the glomerulosa they are narrow, oblong and pointed. At some places they may be found right in to the stroma of the connective tissue capsule, so that they here give the impression of having become differentiated from elements in the stroma (Fig. 3). Herefrom they extend in sector-shaped or belt-like portions inwards to the centre of the organ (Fig. 1). In

the fasciculata they have a more polygonal shape with excavated contours between pointed cytoplasmic offshoots. Here we may find the above-described »starlike« figures (Fig. 4). The morphological picture may be imagined to have been produced through an independent proliferation of the two cell-types. The light-coloured cells may, owing

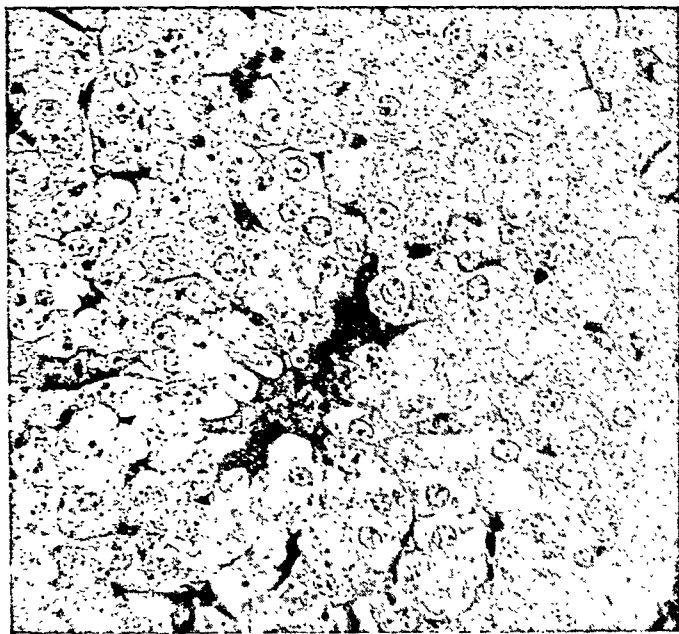


Fig. 4.

Conglomerate of »siderophile« cells in outer fasciculata, forming a »stellate figure« amidst the light cells. Normal adrenal gland.
Haidenhain's ferrohematoxylin. $\times 400$.

to their greater vigour of growth, press the dark-stained cells together, so that they acquire a compressed, excavated appearance.

In the central zone the dark cells predominate, together with the sympathetic elements of the medulla. They are larger than the corresponding cells in the fasciculata. They are polygonal or elongated, especially when they lie close in to the blood sinuses, forming trabecular arrangement around the latter (Fig. 5). The cell boundaries are often not sharply defined. The cytoplasm contains varying quantities of dark-hued granules, as well as some peripheral vacuoles (Fig. 6). The nucleus is mostly large and rounded, poorly supplied with chromatin and light in colour, and has one or two dark nucleoli. The nucleus may extend right out to the cellular membrane, the cytoplasm being, so to speak, constricted at the middle and surrounding the nucleus on both sides like a halo. The light cells which dominate in the fasciculata are present in very small numbers in the central zone (Fig. 5).

The above-described cortical cells with dark granules show in their morphology several points of resemblance to the »siderophile« cells in the adrenal cortex, and must be supposed to be identical with the latter. Dostoiewsky already in 1886 gave an exhaustive account of the »siderophile« cells, and little has since been added to his descrip-

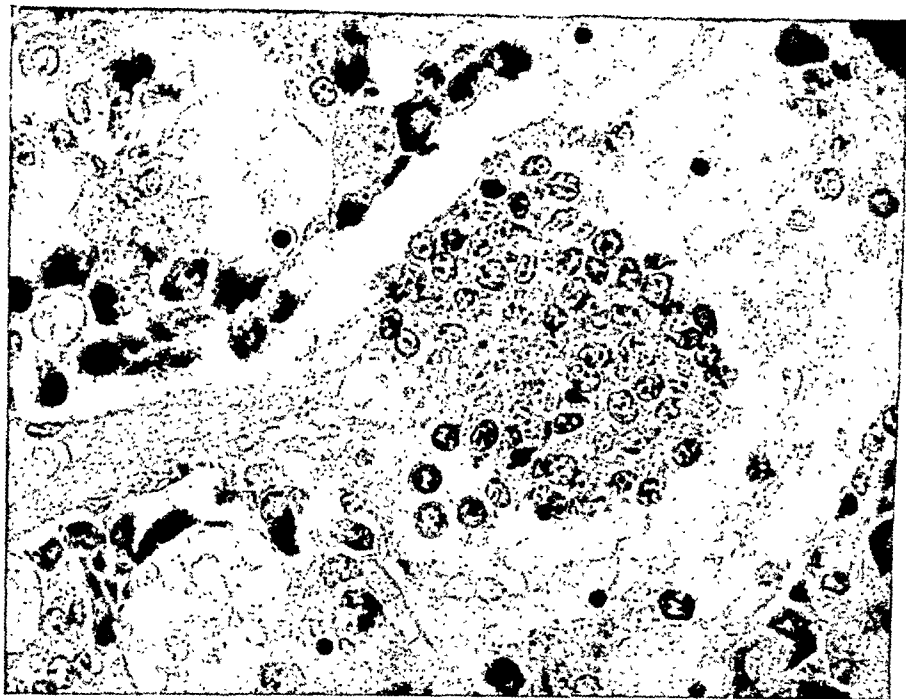


Fig. 5.

Normal adrenal gland, central zone. Large blood sinus bounded by dark-granulated cells. Within the sinus a »rosette« of sympathetic nerve cells connected by nerve fibre.

Haidenhain's ferrohematoxylin. $\times 600$.

tion. The theory that these cells exert a secretory activity is of old date and has been maintained by, among others, Guieysse (1901), Bernard and Bigart (1905), Ciaccio (1905), Mulon (1911), Da Costa (1913). Goormaghtigh (1922), however, has in special degree supplied support for this theory through his searching histological investigations. While most authors regard the »siderophile« cell inclusions as secretory granules, Hoerr (1931) finds nothing to support this view. He introduced the designation »dark and light cells« for cells in the reticularis in guinea-pigs, where some of the cells were of an intensely dark colour, while some were very light-coloured, and he regards both these cell types as forms of incipient degeneration. The histological picture of the cortical cells in our material also accords very well with Hoerr's description of »dark and light cells« and thus gives good occasion for a discussion of the two theories.

The sympathetic cell elements in the central zone are, morphologically regarded, of several types. Sometimes they occur as typical rosettes (Fig. 5), similar to those described by Pankratz in the rat and by Keene and Hower in man (1927). The rosettes are built up of cells with medium-sized nuclei, rich in chromatin and surrounded



Fig. 6.

Normal adrenal gland, transition from fasciculata to central zone. Dark-granulated cells and blood sinuses. Som few light cortical cells.

m: heap of medullary cells. b: blood sinuses.

Haidenhain's ferrohematoxylin. $\times 1000$.

by a faintly stained cytoplasm. These rosettes lie inside in blood rooms and are often connected with nerve fibres which may be seen to run outwards into the cortex, sometimes right out to the capsule. Another type appears as light-coloured cells in heaps or »nests« (Fig. 7) and resembles those described by Waring (1927) in the mouse. These heaps of cells are smaller than the described rosettes and the cells here are larger and very light with large oval nuclei, poor in chromatin, and a very pale cytoplasm (Fig. 6). Such cells are found either in relation to blood sinuses or more often surrounded by darkly granulated cells. They are seldom found in connection with nerve fibres. Cells of this type are also seen in larger or smaller heaps outwards in the fasciculata and glomerulosa, right out to the capsule, as an indication that immigration of cells from the sympathetic ganglia

is still taking place. By their arrangement in »nests« with indistinct cell-boundaries they are distinguished from the surrounding cortical cells. Nerve fibres are found in the central part, as well as in stripes extending radially outwards into the cortical substance. Sometimes they are found to be isolated, sometimes in connection with nerve

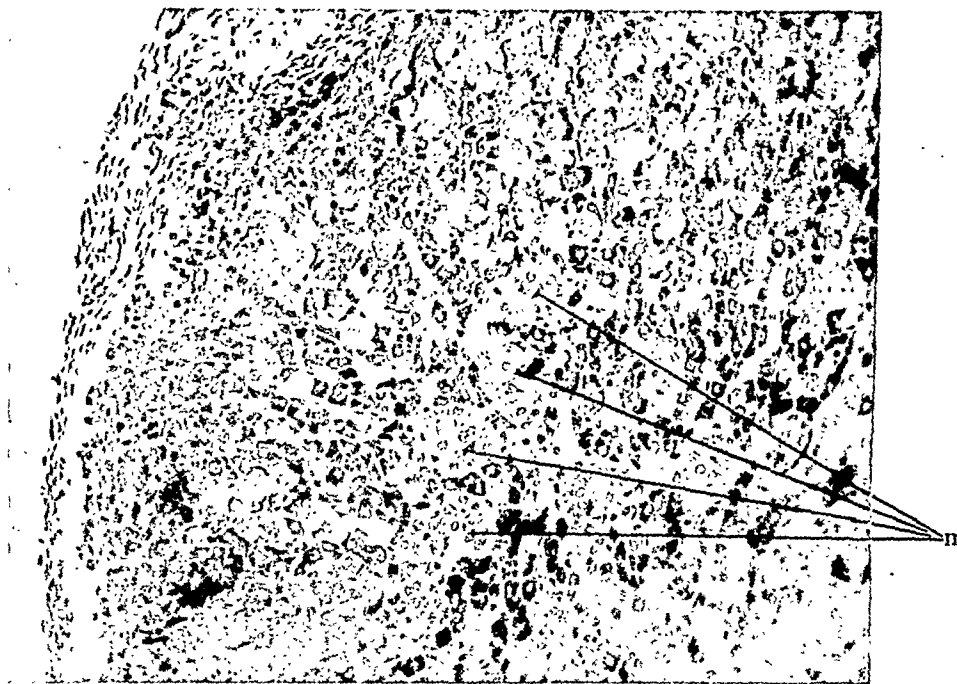


Fig. 7.

Fetal adrenal gland after adrenalectomy. Proliferation of dark-granulated cells from the periphery in through the fasciculata to the central zone.
Few light cortical cells.

m: Heaps of medullary cells in »nests«.
Haidenhain's ferrohematoxylin. $\times 200$.

cells. Mingled with the nerve fibres are found nerve sheath cells, but no typical ganglion cells. Along the nerve fibres and the nerve cells are seen the darkly granulated cortical cells, arranged in one or more rows.

In their morphology the adrenal glands of the new-born rat are still characterized by migration of sympathetic cell elements into the mesenchymal cortex anlage. The differentiation into three topographical zones, such as we find in the adult animal, has not yet been attained. No central medullary zone has been formed, and the fasciculata has not yet acquired its typical appearance.

What gives the organ its special stamp is the building up of the two characteristic cell types in the cortex, the darkly granulated and the light-coloured cells. Further must be emphasized the peculiar cen-

tral zone with its variegated morphological picture of granulated cortical cells surrounding blood sinuses, together with sympathetic cell elements. Comparative investigations of other rodents, especially mice, have revealed the presence of a similar central zone, found during embryonic life and in the time immediately following. These interesting features will therefore be later taken up for discussion when dealing in detail with our material.

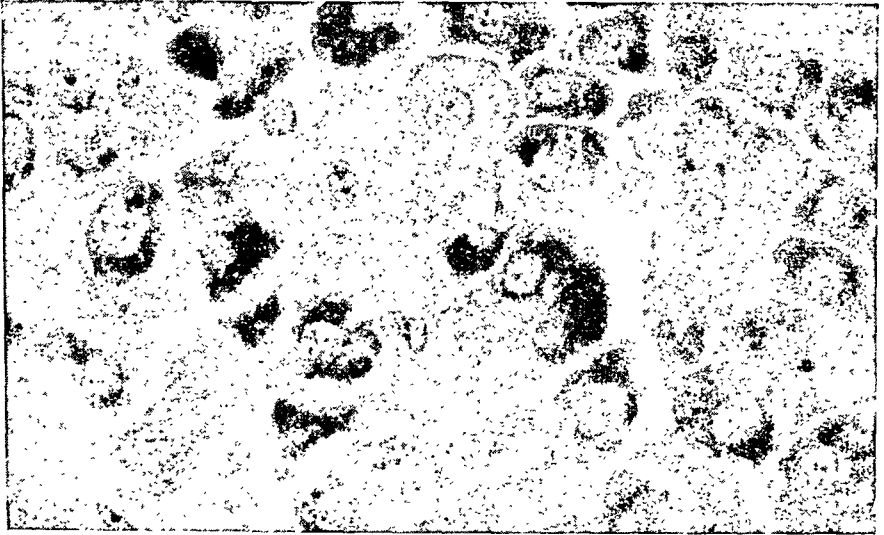


Fig. 8.

Adrenal gland after adrenalectomy. Portion from central zone (detail from Fig. 2). Numerous highly granular cells.
Haidenhain's ferrohematoxylin. $\times 1000$.

b. The adrenalectomized group (Fig. 2).

In its histological construction the zona glomerulosa here shows about the same structure and extent as in the normal adrenal glands. The zona fasciculata is a little broader than normal and shows for the most part a regular picture with large light-coloured cells. Some parts, however, are dominated by large numbers of dark granulated cells with some few light cells in between (Fig. 7). In serial sections it is seen that the dark cells often form irregular whorls, extending from the capsule inwards through the glomerulosa and fasciculata.

Meanwhile the organ is here especially characterized by the great extent of the central zone. On cross-section through the middle part it is seen to represent one half of the diameter of the whole organ. A high degree of hyperaemia is observed and the blood vessels have increased both in number and size. Largely distended irregular blood sinuses, surrounded by dark granulated cells, dominate the picture. These dark cells show a great increase in number as compared with

normal organs. On the other hand, no certain increase in the sympathetic cell elements has been noted.

In their morphological structure the cell types in the glomerulosa and fasciculata seem to differ little from the normal. The dark granulated cells in the central zone present, however, a highly varied picture. They vary greatly in configuration and size. Some of them are large polyhedral cells densely supplied with granules in the cytoplasm (Fig. 8). More frequently are found, however, elongated or pointed cells, often with irregular configuration and ill-defined boundaries. The granulation varies from a homogeneous, highly granulated cytoplasm to a light cytoplasm with hardly any granules. Most of the cells are partially granulated and granules are often found in the periphery of the cytoplasm. Irregularly situated intermedial vacuoles are frequently seen. A special type of cells are in the shape of large rounded polygons, having numerous small vacuoles and few granules in the cytoplasm.

The majority of the cells have a large, pale nucleus with circular or oval contour and a light-coloured chromatin network. The nucleus often extends right out to the membrane of the cell, the cytoplasm being constricted at the middle. The most highly granulated cells often have a somewhat smaller and denser nucleus.

Thus a typical feature of this group of adrenal glands is the hypertrophy of the central zone, as well as the irregular morphological picture here presented, with the polymorphous appearance of the granulated cells.

After this general account of the histology of the adrenal glands we shall discuss the morphological data which the material furnishes for judgment of the functional state. As already mentioned, we shall base this discussion on studies of the mitotic activity and cytological structure of the adrenal glands.

Mitotic activity in the adrenal glands.

The formation of new cells in the adrenal glands takes place in outer parts of the organ, in the zona glomerulosa and the outermost part of the zona fasciculata. Gottschau (1883) was the first to describe a constant reproduction of cells from the outer layer of the adrenal cortex and parallel herewith a destruction and decay of cells in the inner parts of the cortical reticularis.

Subsequent investigators have found mitoses in the outer parts of the cortex corresponding to the germinative layer. For instance, Mulon (1903) found mitotic figures in greatest number 4 to 6 rows of cells in from the capsule: between the zona glomerulosa and the zona fasciculata. Other authors (Canalis 1887, Kolmer 1918, da Costa 1918 and Hett 1926) also assign the localisation of the mitoses to these

germinative zones, while they very seldom find mitoses in the deeper layers of the cortex and never in the medulla.

The constant presence of mitotic figures in the outer layers of the cortex has been thought to indicate a continual process of regeneration from these parts of the organ. The general opinion has been that the cells are constantly being moved inwards towards the centre and that they undergo degeneration in the zona reticularis. Thus Hoerr (1931) found in guinea-pigs that the cells in the zona reticularis often have pycnotic and fragmented nuclei and that the cytoplasm shows degenerative changes in the form of shrinkage and vacuolisation, or conversion into acidophile cell debris. These cellular changes were by earlier investigators in this field conceived as denoting the death of the cortex cells and the conclusion of the cortical cell cycle.

By investigation of the mitotic activity in the adrenal cortex it was thought to get an indication of the functional state of the organ both in normal condition and under the influence of various irritants.

The mitotic activity has been ascertained by counting the mitoses. Earlier investigators (Schmeckebier, Hoerr and others) have by their countings in the adrenal glands of guinea-pigs found that the mitoses are rather evenly distributed through the whole organ, and they believe a count made in three longitudinal sections through the centre thereof must be deemed sufficient to afford a measure for the total number.

Previous works in this field show that in normal animals there is some variation in the mitotic activity. In investigations of the adrenal glands of normal guinea-pigs the following figures have been found:

	Number of mitoses per section	Animal's weight	Thickness of section
Hoerr.....	2-8	300-400 g	3-4 μ
Schmeckebier.....	5	190-200 „	6 „
„	1.5	400-600 „	6 „
Saxton & Green.....	10.2	180-200 „	
Schmidt & Schmidt.....	40		10 „
Blumenthal	4.1	180-200 „	6 „

In one single investigation (Schmidt & Schmidt) there were found 40 mitoses per section, but other authors have been unable to give any explanation for this high figure. From the results recorded by the other investigators it appears that the figures vary a good deal. Thus the number of mitoses is found to decrease with increasing age. Among other factors which may possibly explain some of the varia-

tions observed is the dependence of the number of mitoses on external influences, such as the temperature and the state of nutrition. High temperature has been found to reduce the mitotic activity, whereas cold has a stimulating effect thereon. The importance of the state of nutrition for the mitotic activity has been proved by experiments in under-nourishment, which was found to lead to a reduction in the number of mitoses. These factors, especially the variations in temperature, may be supposed to be responsible for some of the fluctuations observed. But we must also count upon a certain range of variation even when the conditions are otherwise quite similar.

The increased mitotic activity which can be brought about experimentally by stimuli of various kinds gives an easily recordable indication of the organ's hyperactivity. Counting of mitoses has accordingly been a method employed both in investigations of regeneration processes after toxic influences and for demonstration of cortical hyperactivity after various hormonal stimuli.

In earlier investigations rises in the number of mitoses after direct administration of hormones have been recorded. For example, Schmidt & Schmidt (1938) in the adrenal glands of guinea-pigs found a threefold increase in the number of mitoses after ingestion of thyroxin. The mitoses were here found chiefly in the zona fasciculata, not only in the outer part thereof, but also in the deeper layers. These authors believe that the increase in number of mitoses proceeds parallel with the rise in degree of cortical function and gives a more striking indication of hyperactivity than the increase in weight of the adrenal glands which was noted at the same time.

Similar proliferations of the adrenal cortex are found on injection of extracts from the anterior pituitary and after implantation of hypophyses.

Own investigations.

In the present investigation we have recorded the mitotic activity in the fetal adrenals under normal conditions and after adrenalectomy performed on the mother during gestation. As already mentioned, the fetal adrenal glands show an increase in weight after this operation. Investigations respecting the number of mitoses seem well suited to throw light on the question whether this increase in weight represents an actual hyperactivity. In addition to the ordinary counting of the mitoses we have employed the Colchicine method for recording the maximum number of mitoses in the adrenal glands.

The animals were kept under constant conditions as regards food and temperature in order that the mitotic activity should not be influenced by external factors. On counting in total serial sections through the adrenal glands the mitoses are found to be evenly distri-

buted through the whole organ. We have therefore reckoned it sufficient to make a count in 10 central sections ($4\ \mu$ thick) from each gland, and have therefrom calculated the average figure.

On examination of the normal organs the mitoses are found to be localized to the zona glomerulosa and to the outer part of the zona fasciculata, i. e., the portion which has by previous investigators been designated the germinative zone. In the inner layers of the zona fasciculata and in the central zone mitoses are extremely seldom found (only 0,6 per cent of the total number).

The counting gave the following results:

In the normal glands the average number of mitoses was 1,5 per section (Table 2). In the count were included all mitoses in the parenchymatous tissue. Connective tissue mitoses were not taken into account. All stages of cell division have been recorded. The greatest number of mitoses were found in the metaphase stage (67 per cent). Prophases are less frequently found (24 per cent) and only very rarely anaphases and telophases (6 per cent).

The cell forms which by earlier investigators (Hoerr and others) were designated »kinetic nuclei« are found in our material, especially in the zona glomerulosa. These forms are described as cells in which the nucleus is densely filled with small granules and is without any certain nucleolus. The nature of these cells has been disputed. By some authors they have been regarded as pre-prophases and have been included in the counting of mitoses. Other investigators deny that these cells have any connection with the process of cell division. In our material »kinetic nuclei« have been left out of account when counting the mitoses.

In the adrenalectomized group the mitoses have the same localisation as in the normal control organs. There are no indications that the mitotic zone has extended inwards into the deeper layers of the cortex, as noted by previous authors (Schmidt & Schmidt) in their investigations of the adrenal glands in guinea-pigs after ingestion of thyroxin.

Both with respect to their distribution and of their appearance in general the mitoses in this group show great concordance with those found in the normal organs. But the mitotic activity itself differs widely in the two groups.

Whereas the average number of mitoses for the normal animal was 1.5 per section, the average number found in the enlarged adrenals is 4.2 per section (Table 2), i. e., an increase to nearly three times as many. It seems reasonable to regard this rise in mitotic activity as an indication of hyperfunction of the organ, in the same way as the previously mentioned increase in weight. But while the increase in weight may be influenced by other factors than cell proliferation, namely, by oedema, hyperaemia and possible accumulation of secretion, the rise in cellular activity, as directly measured by

Table 2.
Mitotic activity in fetal adrenal glands.

	Number of glands	Number of sections counted	Average number of mitoses per section	Variations in number of mitoses per section	
				max.	min.
Normal control animals	10	100	1.5 (2.4—0.5)	5	0
Adrenalectomized animals	10	100	4.2 (6.7—2.6)	12	1

counting the mitoses, gives a more exact criterion for the existence of an active hypertrophy of the organ.

Investigations of mitoses by the colchicine method.

In order to throw further light on these questions we have made use of the colchicine method for registration of the mitotic activity.

The effect of colchicine on cell division was first demonstrated by Dustin in 1934. He showed that injection of the alkaloid colchicine into an animal arrested the process of cell division in the metaphase stage during the time when the action of colchicine made itself felt in the organism. He found that all cells which entered into the process of division in that period were registered as metaphases in the histological preparation, so that the effects of colchicine could be taken to reveal the mitotic activity. The colchicine itself gave no increased impulse to cell division, which, however, proceeded somewhat atypically.

The process of cell division takes place more rapidly than usual up to the metaphase stage. The metaphase also acquires an atypical appearance. The chromosomes do not show the usual fine structure, but become shorter and thicker than normal, having a clumsy appearance. Often they may clump together and then form the so-called «clumpy colchicine figures». Spindles and centrosomes do not appear distinctly, the equatorial plate may be puffed up, and the whole cell often seems larger than usual and more spherical. Pycnosis is not seldom seen. The prophase stage is rarely observed and then presents a normal aspect. The differentiation of the nuclear reticulum here proceeds in normal manner. The anaphase and telophase stages are practically never seen.

The action of colchicine sets in rapidly after it has been injected. Already after two hours the typical metaphase figures appear.

The effect is greatest after from 6 to 9 hours, the maximum number of mitoses being visible at that time. Afterwards the effects rapidly disappear.

Injection of colchicine has been employed especially in order to demonstrate the mitotic activity in organs in vigorous growth, with proliferation and regeneration. The method has particularly been used in endocrinology as a test of hormonal stimulation.

The colchicine method has been used to investigate the action of estrogens on the mitoses in uterus and vagina (Allen, Gardner & Smith 1937) and of androgens on the mitoses in the prostate (Burkhardt 1939). Leblond and Allen (1937) have likewise by aid of colchicine investigated the mitotic activity in the mammary gland after injection of prolactin.

The technique employed for the colchicine method, and which was also adopted in the present investigation, is as follows:

The colchicine is injected in the animal subcutaneously in a dose of 0.1 mg of colchicine per 100 g body-weight. This dose gives the optimal effect. Smaller doses are not fully effective, while larger quantities are not tolerated, owing to the toxic action of the drug. After 9 hours the organs are removed for examination.

In our investigation the animals were given the above-mentioned dose of colchicine 9 hours before the term. For subcutaneous injection was used an aqueous solution containing 1 mg of colchicine per cc, whereof was injected 0.1 cc per 100 g body-weight. After 9 hours the animals were killed and the fetal adrenal glands were removed for examination. On three of the adrenalectomized animals the colchicine seemed to have had a highly toxic effect and all the fetuses were dead. These animals are not included in the material.

The other animals were not materially intoxicated after injection of colchicine, and the young ones were alive and in vigorous activity at birth.

The counting of mitoses shows the following results:

In the control group the average number of mitoses found was 3.9 per section, while the adrenalectomized group showed an average of 12.4 mitoses per section (Table 3). The ratio between the numbers of mitoses in the two groups is here 1 : 3.4. Thus the greater mitotic activity is very clearly revealed by the colchicine method.

Also here the mitoses are found in the germinative layer, with the same topographical localisation as described for the previously mentioned experimental groups. The mitoses are not seen to extend inwards into the deeper layers of the zona fasciculata. They usually lie in the borderland between the zona glomerulosa and the zona fasciculata, and are often arranged in heaps or rows, with areas free from mitoses in between.

The typical action of colchicine on the appearance of the mitoses is to be seen alike in the normal and in the hypertrophic adrenal

Table 3.

Mitotic activity in fetal adrenal glands.
Mother given injection of *colchicine* 9 hours before autopsy.

	Number of glands	Number of sections counted	Average number of mitoses per section	Variations in number of mitoses per section	
				Max.	Min.
Normal control animals	10	100	3.9 (7.6—2.3)	10	0
Adrenalectomized animals	10	100	12.4 (17.2—8.9)	31	5
Adrenalectomy + Desoxycortico- sterone-acetate injections	10	100	3.4 (5.7—1.0)	8	0

glands. The cells which are in process of division are large, round and pale, often showing dispersion or clumping together of the atypical chromosomes. The metaphase stage dominates in all sections (90 per cent). Where the other stages of cell division can now and then be seen they are of normal appearance.

From the results noted the *colchicine* method seems to give a striking expression of the cortical hyperactivity after adrenalectomy in our material. The accumulation of mitoses that occurs under the influence of *colchicine* should be supposed to give fully as accurate a result as the counting of mitoses alone. Usually the process of cell division is reckoned to take from half an hour to two hours, and such a relatively large variation in time might be thought to lead to a certain degree of inaccuracy in the result of the count. This factor is eliminated in the organs influenced by *colchicine*, where the cell division is arrested and all cells from the pre-prophase stage are registered.

The mitotic activity in the adrenal glands after administration of large doses of adrenal cortex hormone has also been investigated. The animals were adrenalectomized on the 16th day of gestation. Afterwards 5 mg of desoxy-corticosterone-acetate »Bayer« was injected twice daily until term and the animals were treated with *colchicine* as previously described.

The average mitotic activity in these adrenal glands is 3.4 mitoses per section and thus lies at the same level as in the normal adrenals after treatment with *colchicine* (Table 3). The artificial administration of cortical hormone has thus reduced the number of mitoses

to the normal figure. The increased mitotic activity after adrenalectomy must therefore be assumed to stand in specific relationship to the hormone deficit and the thereby produced metabolic changes.

Cytology of adrenal glands in relation to secretory functions.

As pointed out in the general description of the adrenal glands in our material, two typical and essentially different forms of cortical cells occur. On the one hand we find large, light-coloured cells rich in lipoids, on the other hand smaller, oblong, dark-staining granulated cells. Also other authors have found cell types with the same appearance in the adrenals. One of the central problems concerning the cytology of the adrenal glands is the nature of these cells. Are they to be regarded as the same type of cell in different functional stages, or are they two separate cell types differing essentially in morphology and functions? The subsequent discussion of our material is intended as an attempt to throw light upon this fundamental problem. In particular we shall deal with the cytology of the granulated cells. As already mentioned, opinions differ as to the nature of these cells. Goormaghtigh and others on the basis of their cytological studies have ascribed to them secretory functions, whereas Hoerr regards them as degenerative forms. In the following account we shall therefore have in mind the possibilities named:

- 1) Do the granulated cells show morphological signs of degeneration?
- 2) Is there any evidence of secretory functions in these cells?

1) That the cells observed in our material are identical with Dostoiewsky's »siderophile« cells and with Hoerr's »dark and light cells« seems to be beyond doubt. From Dostoiewsky's detailed description (1886) we shall here quote: »Stellenweise ordnen sich dieselben (light cells) in regelmässigen Reihen an, sind gut contouriert, enthalten einen Kern mit deutlich sichtbaren Kernkörperchen. Das Protoplasma ist feinkörnig. In anderen Fällen ordnen sich Complexe von Zellen an, die in regressiver Metamorphose begriffen zu sein scheinen, sie werden kleiner und in ihrem Protoplasma zeigen sich von allen Seiten Einkerbungen, so dass die Zellen eine sternförmige Gestalt annehmen. Eine geringe Menge Protoplasma bleibt nur um den Kern herum. Die Zellen grenzen sich nicht deutlich von einander ab, und scheinen mit ihren Fortsätzen zu verschmelzen, so dass die Rindensubstanz auf Schnitten dieser Stellen gleichsam ein feines Gespinnst darbietet.« This description could apply completely to the cells found in our material.

Da Costa (1913) pointed out that the term »siderophilia« comprises two types of cytoplasmic components: Some cells contain distinctly siderophile granules, while others acquire a diffused dark colouring in the cytoplasm on staining with iron-hematoxylin.

Hoerr (1931 and 1936) has described the cell types found in the

adrenal cortex of guinea-pigs. He introduced the designation »dark and light cells« for types of cells seen in the reticularis. »Light cells« are described as round, spherical cells with finely granulated cytoplasm, retracted from the cellular membrane. The extremely fine granules are seen to be dispersed through the cell and have little affinity for stains. The nucleus is large, vesicular and pale-coloured. »Dark cells« are characterized by their intensely stainable cytoplasm, which stains black with iron-hematoxylin. After staining the cytoplasm appears firm, homogeneous and almost »vitreous«. It becomes difficult to distinguish between mitochondria, pigment, granules and lipid droplets in the dark cytoplasm, which stains intensely in its entirety. These latter cells thus show a marked siderophile reaction in the cytoplasm.

Hoerr in his monograph deals with the siderophile staining reaction in general and points out that there is here no question of a histochemical reaction. The siderophile reaction is not due to any specific chemical substance in the cell, but is assumed to depend upon a certain physical state which produces an intense affinity to hematoxylin. Hoerr supposes that this reaction is to be observed especially in cells in incipient autolysis owing to changes in the colloids. These cells would thus represent an incipient stage of degeneration. Hoerr has also shown that the siderophile staining may arise through imperfect fixation, as an artefact. Many of the earlier described siderophile substances are therefore presumed to be due to bad fixation, or to autolytic changes occurring in the cells before fixation has taken place.

Hoerr denies the possibility that the siderophile substances may be products of secretion: »It is extremely improbable that the siderophile formations represent stages of a secretory cycle, or that they are constant products of cellular metabolism.« Hoerr's conclusion is therefore as follows: »The dark and light cells are senescent cells destined soon to degenerate.« He bases this assumption partly on the localisation of the cells to the reticularis and their occurrence here together with degenerative cell forms, partly on his investigations respecting the siderophile staining reaction, which he regards as typical for autolytic or degenerated cells.

Meanwhile, an investigation of the siderophile cells in our material furnishes no support for this theory. The siderophile reaction in the cytoplasm occurs chiefly in distinct, well-defined granules. In some of the siderophile cells the cytoplasm has moreover a diffused dark-coloured basal substance.

In our preparations these cells are found in all three zones of the adrenal gland, in the glomerulosa, the fasciculata and the central zone. They are seen right out at the capsule in intimate contact with the stroma cells, apparently as a differentiation therefrom. We have also been able to observe mitoses in the siderophile cells on the

boundary between the glomerulosa and the fasciculata. These findings thus seem to indicate an active proliferation of the siderophile cells from the germinative layer. The cells stand in strong contrast to the light cells in the glomerulosa and fasciculata, and transitional forms between the two types of cells are never seen. The mesenchymal elements in the germinative layer accordingly seem to be differentiated in two different directions:

1. To large, round, light-coloured cells rich in lipoids.
2. To smaller, irregularly formed, greatly granulated cells — identical with the siderophile type.

As already mentioned, these cells occur in the germinative zone and have a well-preserved cytological structure, without signs of degenerative changes. These circumstances thus speak against the assumption that we have here degenerative cells. Hoerr has been able to detect none or only very few of these types of cells in the outer zones of the adrenal glands in guinea-pigs. The explanation may be that he was investigating adult animals, in which the localisation of such cells is different. Our investigations of full-grown rats (unpublished work) likewise seem to point to a different localisation of the siderophile cells than at birth.

As previously stated, the granulated cells in our material are found to be localized especially to the central zone, while the light cortical cells are here very rarely seen. The normal degeneration of adrenal cortex cells is not to be seen in our preparations. Hoerr finds in the reticularis necrotic cells, cell debris, macrophages and other phagocytary elements together with the siderophile cells. On examination of serial sections from normal adrenal glands in our material we have not found such signs of degeneration. In the fetal adrenal glands which are still rapidly increasing in size one would also expect to find the normal process of degeneration little in evidence. The granulated cells in this central zone are morphologically somewhat different from the same type of cell in the outer zones of the cortex. This can, however, be explained through the close relation of these cells to the blood sinuses and the heaps of sympathetico-chromaffin cells in the centre, while the cells in the periphery seem to be compressed between the light cortical cells.

Our material accordingly furnishes no evidence in support of the view that the siderophile or granulated cells are degenerative forms. Hoerr's assumption that this siderophile reaction is exclusively associated with autolytic processes is not very probable either. As already stated, the reaction is not specifically histochemical. Neither can it well, we suppose, be associated with a definite pathological process.

Thus it seems from our investigations that the cells of the adrenal cortex are primarily differentiated in two directions, with formation of two morphologically differing types of cells: the light-coloured,

non-granular, and the dark-coloured, granular cells. Both of these types proliferate in the germinative layer, where mitoses appear, and afterwards the cells migrate inwards. The light cells form the greater part of the zona fasciculata, while the dark cells are specially localized to the central zone. No signs of degeneration have been noted in the cells. Whether the difference in morphology is also indicative of a difference in function cannot be decided from these investigations alone.

2) The morphology of the adrenal cortex cells in man in relation to its secretory activity receives special attention from Goormaghtigh (1922). In the juxtamedullary zone, which represents one-eighth of the whole cortex, he finds cells characterized by their content of granules staining intensely with iron-hematoxylin, and which for the most part are reducing osmic acid. The siderophile granules he supposes to be formed by an accumulation of mitochondria at one pole of the cell. The granules gradually come to be surrounded by a colourless halo, an irregular vacuole is formed and the granules disappear. During this process the cell acquires a very irregular appearance owing to its varying content of granules and vacuoles. These morphological changes are regarded by Goormaghtigh as stages in a secretory cycle.

Especially valuable investigations respecting the secretory function of the adrenal glands have been carried out by Broster & Vines (1933 and 1938). In clinical conditions of virilism, brought about by hyperplasia or tumours in the adrenal cortex, they have been able to demonstrate a special type of cell therein. By an acid-fuchsin staining method (the Ponceau fuchsin method) they have here revealed cells of which the cytoplasm stains intensely with acid-fuchsin. In normal adrenal glands, or in indifferent or feminizing tumours, this type of cell is not to be found or is present in very small numbers. These cells are therefore presumed to stand in a specific relation to the increased production of androgens in the adrenal gland in such conditions, and their morphology with occurrence of »fuchsinophile« granules in the cytoplasm, is regarded as expressive of secretory activity. Vines has also established the interesting fact that such cells are normally to be found at a definite period of fetal life in man. Simpson & Joll (1938) and Goormaghtigh (1940) have likewise in virilizing tumours in the adrenal cortex found cells which stain »siderophile« with ferrohemaotoxylin and »fuchsinophile« with acid-fuchsin. These affinities for particular stains are here due to the occurrence of granules in the cytoplasm, and in their cytology these cells resemble the »siderophile« cells described in Goormaghtigh's earlier works.

Thus from studies of the cytology of the granular cells found in the adrenal cortex we obtain some support for the view that these cells have secretory function.

The fetal adrenal glands in our material contain, as already stated,

precisely such granulated cells, staining »siderophile« with ferro-hematoxylin and »fuchsinophile« with acid-fuchsin. The number of the granules, and thereby the intensity of staining of the cytoplasm, varies a good deal, so that the cells present a somewhat irregular morphological picture. Morphologically they show resemblance to the granular cells which by the before-mentioned authors have been regarded as associated with secretory activity. When discussing the cytology of these cells we will examine whether also our investigations furnish evidence of secretory specificity in these cells.

The siderophile or fuchsinophile granules are best seen after fixation in chromic-acid (Flemming's solution) or chromic-acid-formol. After fixation in ordinary formol or potassium bichromate-formol mixtures (Müller-Formol or Zenker) the granules do not come out distinctly in the staining, and the typical stain-affinities are partly lacking.

The granulated cells are found to be specially localized to the central zone. They are also seen, however, in the glomerulosa and fasciculata, but here, as already mentioned, in considerably smaller number. In the normal organs the cells show a fairly uniform granulation and have only some few peripheral vacuoles in the cytoplasm. The nuclei are large and pale, with uniform appearance. The shape of the cells varies somewhat, and especially in the central part, where they are in contact with blood sinuses, the configuration is irregular.

In the adrenalectomized group there is found a great increase in the number of these cells in the central zone of the glands. Morphologically they also show a more irregular appearance. The size and shape of the cells and their content of granules and vacuoles vary greatly. The granulation varies from cells with numerous, large granules in the cytoplasm to light cells almost without granules, and all intermediate stages between these types are to be seen. Vacuoles occur frequently. Most of the cells have small, irregular vacuoles, situated intermedially or peripherally in the cytoplasm. Some of the cells are densely pervaded by small vacuoles through the whole cytoplasm, and have only a few granules. The nuclei are most often large and pale. In irregularly configured cells the nucleus may also have an irregular shape.

In this adrenalectomized group we have previously been able to note a rise in weight, as well as an increased mitotic activity in the glands. Both these findings point to an increase in functional activity. As already mentioned, also earlier physiological investigations speak in favour of a hyperactivity of the fetal adrenals after adrenalectomy performed on the mother during gestation. The above-described morphological picture of the glands can also accord with a state of hyperactivity. There is seen hyperaemia in the central zone, with an increase in the extent of that zone. Moreover there is found an increase in the number of granulated cells therein, together with an

irregular morphological picture and the presence of large numbers of granules in the cytoplasm.

Also for other endocrine organs the content of granules in the cytoplasm has been regarded as having relation to secretory activity. As regards the hypophysis Severinghaus (1939) states: »The elaboration, storage and loss of granules are all to be regarded as phases

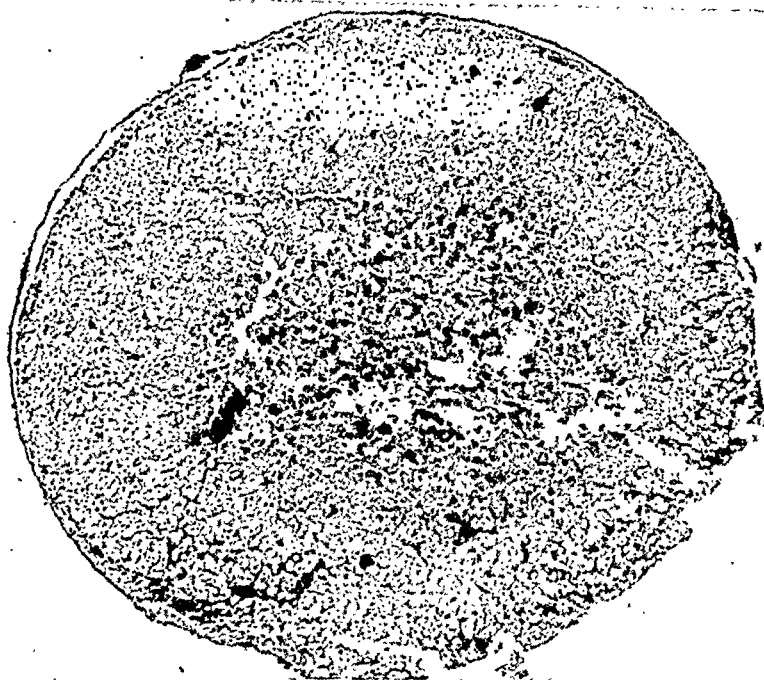


Fig. 9.

Adrenalectomy + injection of desoxy-corticosterone acetate. Broad fasciculate with numerous light cells. Central zone of moderate size. Few granular cells.

Cf. Fig. 2.

Haidenhain's ferrohematoxylin. $\times 60$.

of secretory activity, and any proportional shift in the cells becomes an indicator of the gland's secretory significance. Here also lies the possibility of associating specific hormones produced by the gland with particular cell types.« Also other constituents of the cell, such as nucleus, mitochondria and Golgi apparatus, show variations in relation to the secretory function. An irregular morphological picture, with greatly varying content of granules in the cytoplasm, is found in these investigations of the hypophysis in states of intense secretory activity. Precisely such a morphological picture is characteristic for the fetal adrenal glands in the adrenalectomized group, as has been mentioned above. These investigations respecting the cytology of the hypophysis in relation to secretory activity accordingly

also support our supposition of hyperactivity in the fetal adrenal glands after adrenalectomy.

The deficient production of adrenal cortex hormones after adrenalectomy, and the metabolic changes occasioned thereby, must be assumed to be the incitement which stimulates the fetal adrenal glands

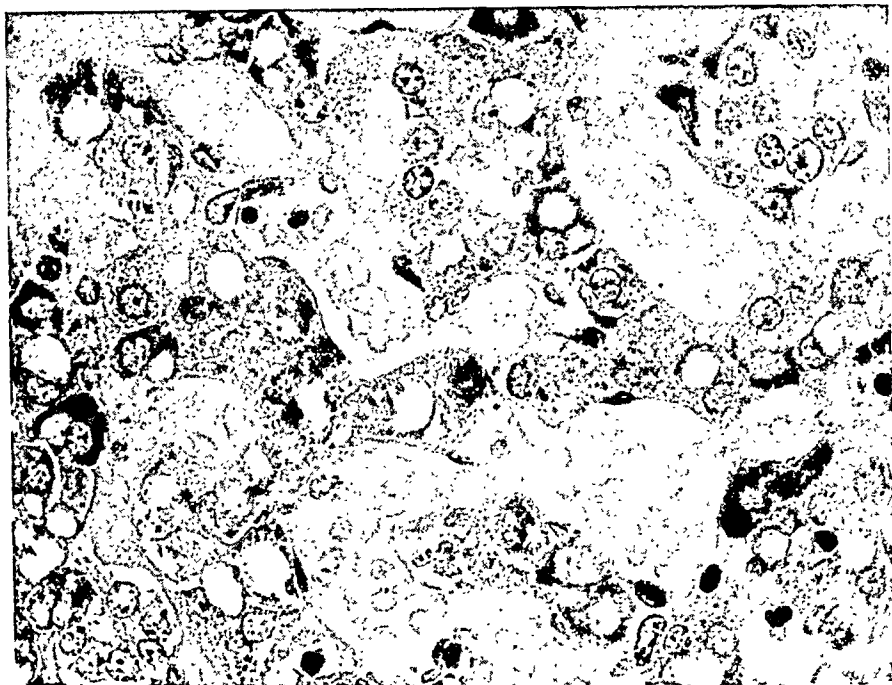


Fig. 10.

Portion from central zone, hormone-treated animal. Trabecular arrangement of vacuolized cells around blood sinuses.

Haidenhain's ferrohematoxylin. $\times 600$.

to increased functional effort. In order to confirm this assumption of a hyperactivity which we arrived at from our morphological investigations we have investigated the cytology of the granulated cells after administration of large doses of adrenal cortex hormones.

Two animals were adrenalectomized on the 16th day of pregnancy and were afterwards given — daily 10 mg of desoxycorticosteroneacetate »Bayer«, in two injections, morning and evening. At term the usual post mortem examination was made.

The fetal adrenal glands here present a compact, broad fasciculata, constructed of large, light-coloured cells with abundant content of lipoids in the cytoplasm (Fig. 9). The central zone, on the other hand, is considerably reduced in comparison with the extension of this zone in the adrenalectomized group. There is complete absence of hyperaemia and the cells contain few granules in the cytoplasm.

In some of the glands the aspect of the central zone is somewhat different, with presence of numerous vacuoles in the cytoplasm of the cells (Fig. 10). The vacuoles are most often found dispersed in the periphery or in the intermediary parts of the cytoplasm, separated from each other by scanty numbers of granules. In other cells these

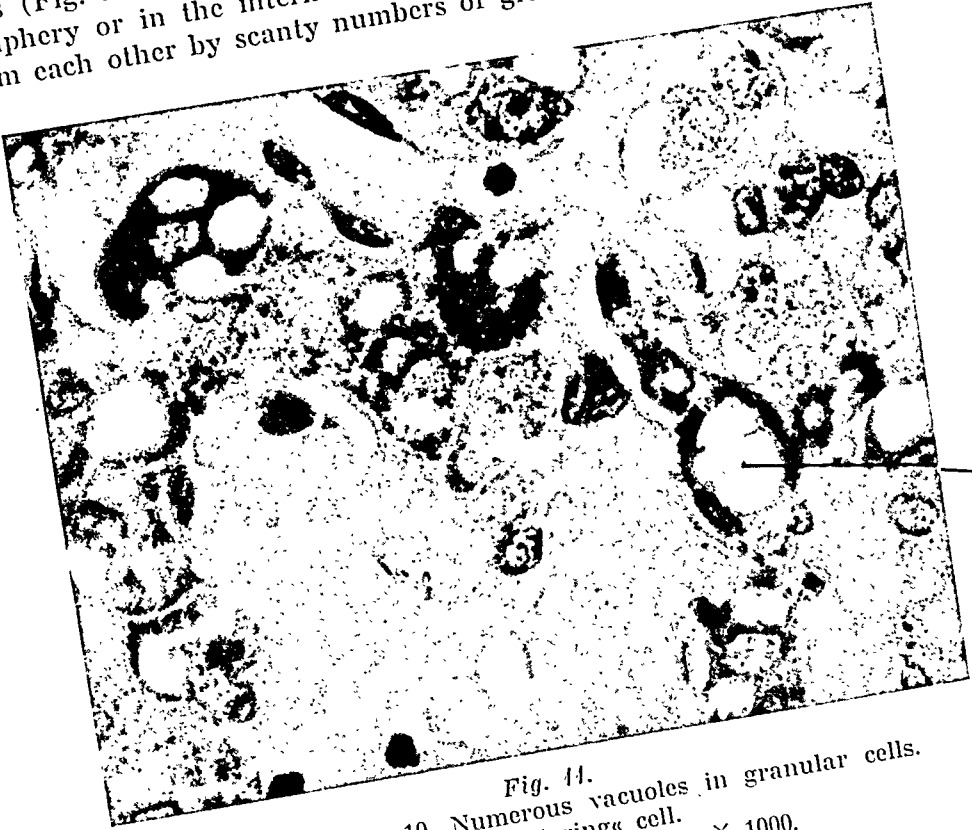


Fig. 11.
Detail from Fig. 10. Numerous vacuoles in granular cells.
s: »Signet ring« cell.
Haidenhain's ferrohematoxylin. $\times 1000$.

vacuoles are confluent. The vacuoles may occupy the whole of the cytoplasm, and the nucleus is pressed against the cellular membrane, acquiring an oblong, pycnotic form. The granules have then quite disappeared from the cell. These cells show a great resemblance to the »signet-ring« cells seen in the hypophysis (Fig. 11). Some vacuoles may also be seen lying free in the capillaries between the blood corpuscles.

After administration of large doses of synthetic adrenal cortex hormone to the adrenalectomized animals there is thus seen a reduction in the extent of the central zone and a disappearance of granules from the cytoplasm. Under these circumstances we must assume from the histological picture an inhibited functioning of the fetal adrenal glands. The central zone and the granulated cells therein thus seem to stand in specific relation to the secretory function of

the adrenal cortex. After adrenalectomy with reduction of the production of hormones in the adrenal cortex of the mother there sets in a hypertrophy of the cellular elements in the central zone of the fetal adrenal glands with morphological signs of hyperactivity. On injection of synthetic adrenal cortex hormones the central zone is again reduced in extent. This is probably indicative of reduced secretory activity.

As before mentioned, numerous vacuoles are found in the cytoplasm of some of the glands. The rôle played by the vacuoles in the cycle of a secretory cell has not been quite clearly established. From their investigations respecting the secretion of the hypophysis Severinghaus, and also Baillif assume that the vacuoles are associated with the discharge of secretion from the cell. The occurrence of large numbers of vacuoles may therefore be assumed to have relation to a restricted discharge of secretion. Thus these findings likewise speak for an inhibition of secretory functions. The possibility that the vacuoles are expressive of a process of degeneration may also be imagined. One single adrenal gland actually showed a diffused degeneration of the central zone, with indistinct cell contours and new-formation of connective tissue.

The cytological investigations support the assumption that the »siderophile« or »fuchsinophile« cells in the adrenal cortex have relation to its internal-secretory functions.

Morphology and functioning of the central zone.

In the foregoing we have repeatedly made special mention of the central zone in the fetal adrenal glands and its peculiar morphology. Comparatively investigations have made it evident that a similar zone is also to be found in other species of animals. Waring (1936) has studied the development of the adrenal cortex in the mouse and he finds in the embryonic life an inner eosinophile cortical zone, interspersed with medullary elements. This he calls »interlocking zone«. The occurrence of this zone is confined to the embryonic period and the first months of life after birth. Afterwards degeneration of the cells sets in. The reticular zone in the adrenal gland of the adult animal is therefore not forced from cells in this central, »interlocking« zone. The reticularis is formed through differentiation of the cells in the inner part of the fasciculata at a later point of time, when the degeneration of the central zone has already begun.

Waring states that a similar situation has been observed in cats and rabbits. In the cat there is found at birth (Davies: Unpublished work 1931—32) an inner cortical zone interspersed with medullary cells. It gradually degenerates in the first months after birth and then disappears entirely. Roaf (1935) has described a similar zone in the rabbit. Also in these cases the reticularis is not differentiated

until the central zone has degenerated. Waring sums up as follows: »In all these cases the tissue is highly eosinophile, has highly granular cytoplasm and interlocks through its existence with the medulla. There can be little doubt that the three structures are homologous. In the cat it is described as syncytial in nature, and in the mouse the cell boundaries are inconstant in appearance. The separate identity of reticular and interlocking zones is beyond question.«

Waring finds that the »interlocking« zone in the mouse diminishes in extent during the first week after birth. On the 10th day of life it constitutes a border of highly eosinophile cells along the outside of the medulla, which has now been formed. These eosinophile elements are identical with the X-zone in the mouse, first described by Masui & Tamura (1926) and later in more detail by Howard-Miller (1927) and Deansly (1928). The cells in the X-zone is according to Howard-Miller characterized by its dark, finely-granular cytoplasm, and the cells are narrow and flattened, having a compressed appearance. They are irregularly arranged and are intermingled with medullary elements in the boundary zone. The zone is formed in the second week of life after birth and increases in size up to the third or fourth week. In the male it then soon degenerates, but persists in the female and does not degenerate until the close of the reproductive period.

Howard-Miller has in the rat (1938) found a zone in the adrenal cortex analogous to the X-zone in the mouse, and she called it the »juvenile cortex«. Morphologically the cells here are identical with those of the X-zone. The zone appears in the second week of life and increases in size until the fourth week, when degeneration sets in, and there then remains a layer of connective tissue. The zone does not, however, attain the same extension as in the mouse. Neither is the zona reticularis in the adult rat formed from these »juvenile« cellular elements.

The central zone in the fetal adrenal glands of our rats seems to be identical with the »interlocking zone« in the mouse. These centrally located cortical cells are found together with sympathetic medullary elements. The cytoplasm contains granula with marked affinities for staining. Homologous with what is seen in the mouse, cat and rabbit we can also in the rat find a central interlocking zone. In analogy with the findings in mice we have also here found (unpublished work) that this zone diminishes in the first week of life, owing to shrinkage of the cells. A real medullary zone then appears, while the central cortical elements now form a narrow ribbon-like zone outside thereof. Here the cells resemble in their morphology those of the X-zone. They are flattened and compressed and have a very darkly stained cytoplasm with large floccular granules. The central cortical zone in fetal life thus constitutes the point of origin for the »juvenile« cortical cells in the rat.

In our material we have been able to note a marked hypertrophy of this fetal central zone after adrenalectomy performed on the mother. We have also been able to associate this hypertrophy with hyperfunction of the fetal adrenal glands. It must therefore be assumed that this central zone also plays a functional rôle in the fetal life. When great demands are made upon the functions of the fetal adrenals the secretory cells here undergo hypertrophy, probably with increased production of hormones.

The X-zone in the mouse is in special degree influenced by the androgenic hormones. Martin (1930) and Starkey & Smith (1938) have noted a degeneration of the zone after injection of androgens. The effect of corticosterone has not been quite clearly established. Grollmann (1939) finds degenerative changes after injection of corticosterone, whereas Howard (1940) has not been able to detect any changes.

Our experiments with injection of desoxy-corticosterone reveal marked changes in the central zone and the cells there. The zone is reduced in size and the cells are small and contain few granules. Sometimes vacuoles appear in the cytoplasm, together with degenerative changes. The effect seems to be similar to what Grollmann found in the X-zone.

Our investigations accordingly show the following results:

The central zone in the fetal adrenal glands of rats is in its structure and morphology identical with the »interlocking« zone in other rodents. The central zone forms the point of origin for the X-zone or the »juvenile cortex« in the rat. After adrenalectomy performed on the mother during pregnancy this zone hypertrophies. After injection of synthetic adrenal cortex hormone, however, the zone diminishes in extent and degenerative changes occur in the cells. It is therefore probable that the cortical cells of the central zone have some specific relation with the production of hormones in the adrenal cortex during fetal life.

Summary and conclusions.

The present work embraces an investigation of the fetal adrenal glands in the albino rat and their reaction to a failure of the functions of the adrenal cortex in the maternal organism. The hormone deficit is brought about by adrenalectomy in the last third part of the gestation period. Under these conditions there has been found a hypertrophy of the fetal adrenals amounting to 33 per cent, established by weighing the glands at term.

Further there have been made histological investigations of the glands in order to ascertain whether this increase in weight may be regarded as having relation to a functional »compensatory hyper-

trophy». Especially the mitotic activity and the cytological structure of the adrenal glands have been subjected to investigation.

The mitotic activity was ascertained by counting the mitoses, which showed a threefold increase in the enlarged glands. Through accumulation of mitoses, occasioned by injection of colchicine, this increase was made still more clearly evident. The increase of mitoses in the adrenal cortex gives expression for a real hypertrophy and speaks for the existence of cortical hyperactivity. Artificial administration of adrenal cortex hormone to the adrenalectomized animals leads to a reduction of the number of mitoses to normal value. Thus the deficient production of hormones after adrenalectomy seems to be the specific exciting cause of the increase in mitotic activity.

In their histological structure the fetal adrenal glands show a somewhat different picture than in the full-grown rat. The outer zones, the glomerulosa and fasciculata, have an embryonic character and have not attained their typical structure. The reticular zone has not yet been formed. Especially interesting features are seen in the central zone. Here is found a mingling of cortical cells and medullary elements around the central blood sinuses. No isolated medullary zone has yet been formed and immigration of sympathetic medullary cells into the masses of cortical cells is still taking place.

Already at this time the cortical cells show differentiation into two different morphological types. Some of the cells are large, regular and homogenous, with faintly staining cytoplasm. In contrast to these are found cells of irregular configuration with granular cytoplasm, with strong affinity to strains. Both types occur in all zones of the gland. The fasciculata, however, is built up especially of light-coloured cells, while those in the central zone are mostly granular.

After adrenalectomy the gland shows a somewhat altered structure. The fasciculata with its light-coloured cells is moderately enlarged. The peculiar central zone, however, shows the greatest changes. The zone is considerably enlarged, with great hyperaemia and an increase in number of the granular cells around the large, distended blood sinuses. On the other hand the number of medullary elements seems to be unaltered. The granular cells have a greatly varying content of secretory granules in the cytoplasm, and these cytological characteristics speak for an increased secretory activity.

Furthermore, the effects of corticosterone on the fetuses of the adrenalectomized animals have been investigated. The central zone is then found to be greatly reduced in size and the granular cells are few in number. At the same time the granules have disappeared from the cytoplasm and vacuoles appear in some of these cells.

The cytological investigations yield support for the assumption of an increased secretory activity in the fetal gland after adrenalectomy. This activity seems to be especially associated with the »secre-

tory» cells of the central zone and is brought about by the hormonal deficit.

Further is discussed the identity of the central zone with the »interlocking« zone in the mouse. Just as this latter develops into the peculiar X-zone, the central zone forms the corresponding »juvenile cortex« in the rat.

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FASC. 5. 1944.

EINAR MUNKSGAARD · KØBENHAVN
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OXIDATION-REDUCTION POTENTIALS IN CULTURES OF ANAEROBIC BACTERIA

By *Jacob Molland, Ph. D.*

Received for publication 5th Dec. 1943).

The functions of living cells and organisms are connected with a series of assimilative and dissimilative processes. The assimilative reactions build up chemical compounds which are dissimilated by respiration, yielding the energy necessary for the functions of life. From a chemical point of view the assimilation is characterised by reductions, the respiration by oxidations. In aerobic respiration, which is accompanied by oxygen consumption, the oxidation is conspicuous. In anaerobic respiration, however, careful chemical and physico-chemical research is necessary to elucidate the oxidation reactions.

In the present paper the respiration of some anaerobic bacteria is studied by means of electrochemical investigations of living cultures. The bacteria studied are partly obligatory anaerobes, partly facultative anaerobes cultivated under anaerobic conditions.

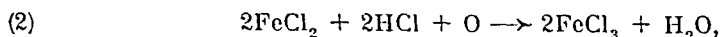
In order to give an exact basis for a discussion of the biological consequences which may be drawn from experiments of this kind, it is necessary to give a survey of the electro-chemical conception of oxidation and reduction.

According to the original definition, an oxidation is a reaction which takes place under oxygen consumption, while a reduction is a reaction which takes place under hydrogen consumption. The simplest case is the formation of water from the elements



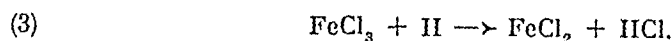
Here the hydrogen is oxidised to water, the oxygen is reduced to water.

It is, however, reasonable to expand the definition of oxidations to comprise also reactions where the oxygen takes part without appearing in the reaction product, e. g. when ferrous chloride is transformed to ferric chloride by means of oxygen:



and to consider ferric chloride as an oxidation product of ferrous chloride.

Analogously the definition of a reduction is widened to include reactions where hydrogen takes part without appearing in the reaction product, e. g. when ferric chloride is transformed to ferrous chloride by means of hydrogen:

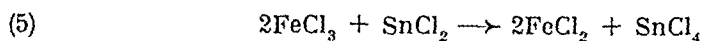


The transformation of ferrous chloride to ferric chloride can, however, be performed without the participation of oxygen:



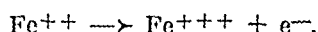
and the definition of oxidations must be expanded to comprise also reactions of this type.

Similarly ferric chloride can be transformed to ferrous chloride without participation of hydrogen:

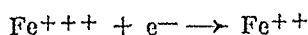


and the definition of a reduction must be widened in like manner.

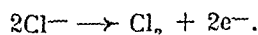
From an electrochemical point of view the characteristic feature of the reactions (2) and (4) is that ferrous ions are transformed to ferric ions by splitting off an electron and thus increasing their electric charge:



The common part of the reactions (3) and (5) is a transformation of ferric ions to ferrous ions by taking up an electron and thus diminishing the electric charge:

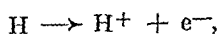


From an electrochemical point of view oxidations consequently may be defined as reactions involving splitting off electrons and thus increasing the positive charge, or numerically decreasing the negative charge:

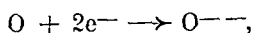


Reductions inversely are defined as reactions involving taking up electrons, thus decreasing the charge.

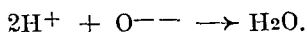
In the reactions (2), (3), (4) and (5) the change of electric charge is conspicuous as the reactions take place between ions. In reaction (1), however, the electric neutral hydrogen reacts with the neutral oxygen, yielding neutral water. But if the reaction is dissolved into its separate links, the change of charge presents itself: Neutral hydrogen is oxidised to hydrogen ions



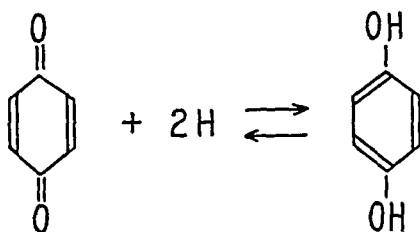
neutral oxygen is reduced to oxygen ions



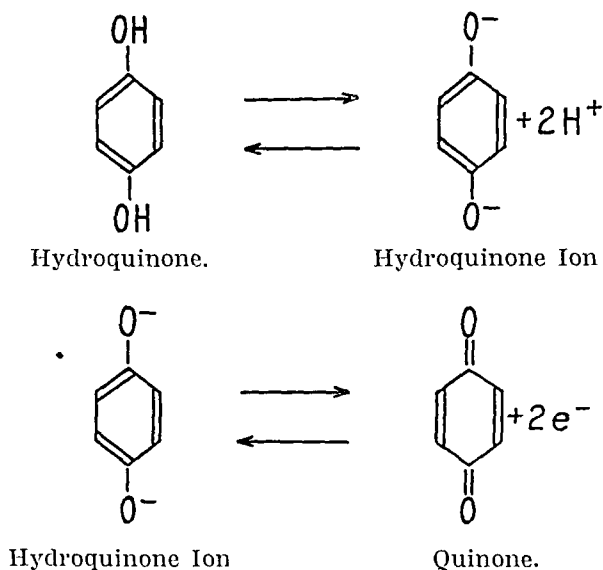
and the ions combine to neutral water



Similarly the oxidation or reduction of other neutral compounds to neutral reaction products can be dissolved into single links where the change of charge is seen. E. g. quinone is reduced to hydroquinone by taking up two hydrogen atoms



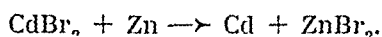
and hydroquinone is readily oxidised to quinone. Hydroquinone is a very weak dibasic acid (dissociation constant $3.10 \cdot 10^{-12}$), and in aqueous solutions there will be present some divalent hydroquinone ions which are oxidised to quinone:



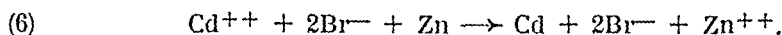
Summarily the reduction of quinone to hydroquinone is a simple taking up of two neutral hydrogen atoms. But the neutral hydrogen atom consists of a hydrogen ion and an electron, and these two are not taken up simultaneously. First two electrons are absorbed, yielding a hydroquinone ion, and it is this part of the reaction which

represents the reduction. Afterwards the hydroquinone ion combines with two hydrogen ions to neutral hydroquinone.

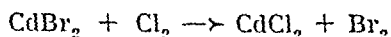
If metallic zinc is added to a solution of cadmium bromide, metallic cadmium will separate:



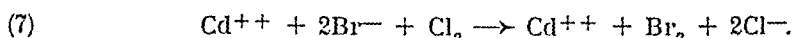
Cadmium bromide here acts as an oxidant, as zinc atoms are oxidised to zinc ions by splitting off two electrons which are taken up by the cadmium ions, reducing them to cadmium atoms:



If, on the other hand, chlorine is added to a solution of cadmium bromide:



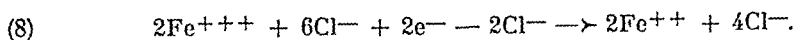
this substance will act as a reductant, as chlorine atoms are reduced to chlorine ions by taking up an electron from the bromine ions which are oxidised to bromine atoms:



Consequently according to the conditions a substance can act as an oxidising or a reducing agent, it has the faculty to give off or to take up electrons. Which of these possibilities will be realised in a given case, depends upon the other component of the reaction. If this component has a greater tendency to take up electrons, the substance will be oxidised, in the opposite case it will be reduced.

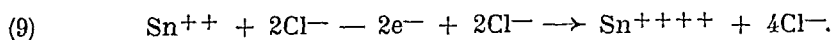
All of the reactions as yet considered have the common feature that oxidation and reduction occur together in the same solution: the electrons which are given off by the substance which is oxidised are taken up by another substance which is reduced. In equation (6) zinc is oxidised while cadmium is reduced, in equation (7) chlorine is reduced and bromine oxidised. This circumstance entails that no electric current is observed as the result of the change of electric charge. By a special experimental arrangement however, it is possible to separate the two reactions, and then an electric current is observed.

Let us consider reaction (5) where ferric chloride is reduced with stannous chloride. What happens is on the one hand that two ferric ions take up one electron each and are reduced to ferrous ions. As a consequence of this, two chlorine ions become superfluous and must be removed if the solution shall be neutral:



On the other hand, a stannous ion gives off two electrons and is

oxidised to a stannic ion. As the charge is increased, the solution must take up two chlorine ions to be kept neutral:



If the two reaction components, ferric chloride and stannous chloride, are kept in two different vessels A and B (Fig. 1), the reaction can be performed if the two vessels are connected in such a way that both electrons and anions can pass from the one vessel

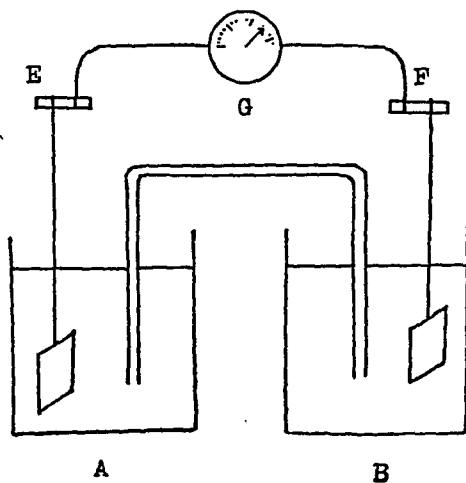


Fig. 1.

to the other. By a suitable experimental arrangement the electrons can be transported by one path, the anions by another. The transmission of the electrons can be realised by a metallic conductor. In each of the vessels is immersed an electrode of platinum or another noble metal, and the two electrodes are connected by a metal wire. The anion transmission is realised through a U-formed glass tube filled with a suitable electrolyte, e. g. a saturated potassium chloride solution. In order to prevent diffusion between the two vessels, two per cent of agar may be added to the electrolyte in the tube. This solution then is converted into a gel which is penetrable for the anions under the influence of the electric forces which arise.

If a ferric chloride solution in the beaker A and a stannous chloride solution in the beaker B are connected in this way, a galvanometer G in the metallic conductor shows the presence of an electric current in the direction from A to B. In the beaker A ferric chloride is reduced to ferrous chloride according to equation (8), in B stannous chloride is oxidised to stannic chloride according to equation (9).

If the current is allowed to pass for some time, a certain quantity of ferric chloride is reduced. If the platinum electrodes are now connected with the poles of an accumulator in such a way that the

current through the circuit is reversed, the chemical reaction will also be reversed: ferrous chloride will be oxidised to ferric chloride at the sacrifice of stannic chloride which is reduced to stannous chloride. The reaction is thus reversible, but the oxidative power of the ferric solution is greater than the oxidative power of the mixture of stannous and stannic ions which is formed, and the reaction spontaneously passes in the direction



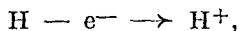
A quantitative measure of the difference of oxidative power between the two solutions is given by the potential difference between the electrodes E and F. If a noble electrode is brought into contact with an oxidising or a reducing solution, there will arise a potential difference between electrode and solution, the so called oxidation-reduction potential. A strongly reducing solution will tend to be oxidised and give off electrons to the electrode which as a consequence gets a negative charge as compared with the solution. The electric field which arises in this way counteracts the passage of electrons from solution to electrode, and an equilibrium is reached when the potential difference between electrode and electrolyte is exactly sufficient to compensate the tendency of the solution to give off electrons. The more reducing is the solution, the more negative will be the potential.

If, on the other hand, the solution is strongly oxidising, it will tend to be reduced and take up electrons from the electrode, and the electrode becomes positive as compared with the solution. The greater is the oxidising power of the solution, the greater will be the potential. When two systems with different oxidation-reduction potentials react, the system with the largest (i. e. most positive) potential will be reduced, the system with the smallest (most negative) potential will be oxidised.

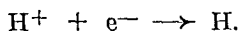
It is, however, not possible to measure a single potential difference between an electrode and an electrolyte. A potential determination always requires two electrodes, and therefore only the difference between two oxidation-reduction potentials can be measured. The oxidation-reduction system to be investigated is connected with a standard electrode, and if the oxidation-reduction potentials of a series of solutions are determined in relation to the same standard electrode, the difference of oxidation-reduction potentials between any of the solutions can be calculated, and it is possible to predict the course of the reaction when the two solutions are mixed, and the energy liberated by the reaction can be calculated.

The standard electrode chosen for theoretical electro-chemical studies is the normal hydrogen cell. This cell consists of a platinum electrode immersed into a normal solution of hydrogen ions. In practice the solution is a 2 N sulphuric acid. A stream of pure hydrogen

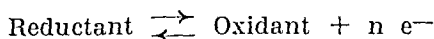
is led through the solution in such a way that the platinum electrode is partly in contact with hydrogen gas, partly with the hydrogen ions of the solution. This cell may act as a reducing agent, the hydrogen atoms being oxidised to ions:



or it may act as an oxidising agent, hydrogen ions being reduced to atoms:



In the case of a reversible reaction, the oxidation-reduction potential can be calculated. Let a reaction be expressed by the equation



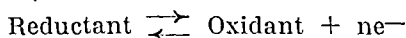
where n is the number of electrons concerned in the reaction. The oxidation-reduction potential E of the system then is expressed by the formula

$$(10) \quad E = E_0 - 0.0001985 \frac{T}{n} \log \frac{[\text{Reductant}]}{[\text{Oxidant}]} \text{ volt}$$

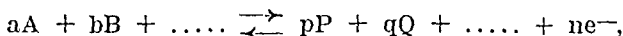
where T is the absolute temperature, $[\text{Reductant}]$ is the molar concentration of the reductant, $[\text{Oxidant}]$ is the molar concentration of the oxidant, and E_0 is a constant depending upon the standard cell used in the estimation.¹⁾ The more the system contains of the reductant, the more negative will the potential be. As the reaction proceeds, the fraction $[\text{Reductant}]: [\text{Oxidant}]$ will be changed, and a corresponding alteration is observed in the oxidation-reduction potential, and when the equilibrium of the reaction is reached, the potential reaches a constant value.

In the present investigation the oxidation-reduction potentials of

¹⁾ If the reaction



is represented by the complete equation



the theoretical deduction of the oxidation-reduction potential gives the formula

$$E = E_0 - \frac{RT}{nF} \cdot \ln \frac{[\text{P}]^p \cdot [\text{Q}]^q \dots}{[\text{A}]^a \cdot [\text{B}]^b \dots}$$

where R is the gas constant, F is one faraday, and \ln is the natural logarithm. Introducing the numerical values of the constants and converting the natural logarithm into the logarithm to the basis 10, and representing the terms $[\text{A}]^a \cdot [\text{B}]^b \dots$ and $[\text{P}]^p \cdot [\text{Q}]^q \dots$ with $[\text{Reductant}]$ and $[\text{Oxidant}]$, the formula can be written in the form (10).

the reactions appearing in cultures of live bacteria are measured. The bacteria are partly obligatory anaerobes: *Clostridium septicum*, *Cl. tetanomorphum*, *Cl. sporogenes*, *Cl. tetani*, *Cl. perfringens*, *Cl. centrismorphum*, *Cl. multifementans* and *Actinomyces necrophorus* (Syn. Necrosis-Bacillus Bang), partly facultative anaerobes cultivated in the absence of oxygen: *Proteus vulgaris*, *Escherichia coli* and *Staphylococcus aureus*. The bacteria were cultivated at 37°, and the oxidation-reduction potentials were measured during a period of 64 to 94 hours.

The apparatus described in the following was built up for the present purpose. It represents an improvement on the usual technique of oxidation-reduction potential estimations, and is adopted for determinations in bacterial cultures which must be protected against infection during the experiment.

A difficulty which arises in the experimental estimations of oxidation-reduction potentials is that some electrodes give irregular potentials. If measurements of the same solution are made with a series of electrodes of the same material, e. g. blank platinum, it sometimes happens that one or more of the electrodes give potentials which deviate considerable from the values obtained by the other electrodes. This deviation may be due to poisoning of the electrode. The poisoning does not manifest itself on visual inspection of the electrode. It is therefore necessary to control the potential of the electrode before use and ascertain that the electrode is sound. The five platinum electrodes used for the present estimations gave the following values in a control experiment:

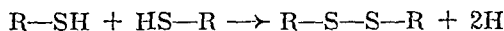
Table 1.
Control of the platinum electrodes.

Elec- trode	Potential	Deviation from the mean value
1	0.3873 volt	—0.0002 volt
2	0.3873 »	—0.0002 »
3	0.3868 »	+0.0003 »
4	0.3873 »	—0.0002 »
5	0.3868 »	+0.0003 »

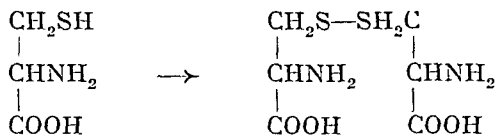
The difference between any of the potentials does not exceed 0.5 millivolt.

As to the choice of electrode material, conflicting results are found in the literature. Dixon and Quastel (1) investigated the oxidation-reduction potential of cysteine solutions, and observed that when platinum electrodes were used, the potentials showed continuous and extensive drifts, and it was not possible to obtain high accuracy of measurement. Substituting the platinum electrode with a gold electrode, however, these authors obtained satisfactory results. In con-

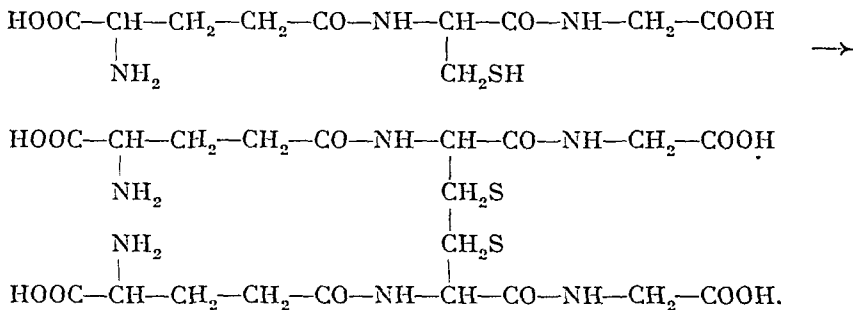
trast to the experiences of Dixon and Quastel, it was found by *Michaelis* and *Flexner* (2) that when oxygen is completely excluded, constant potentials were obtained with electrodes of platinum and of mercury. Only gold electrodes showed individual variations. Whilst electrodes of blank platinum require several hours to give constant potentials, the mercury electrode gives constant values within a considerably shorter time, and the potentials of these electrodes are much less sensitive to traces of oxygen than the platinum electrodes. When oxygen is present, even in minute quantities, the potential of the platinum electrode is displaced in a positive direction. In a later publication, (3) however, *Barron*, *Flexner* and *Michaelis* found that mercury electrodes cannot be used for oxidation-reduction potential estimations of solutions which contain sulphydryl groups ($-\text{SH}$), and this is just the case in the bacterial media, and the respiration of the bacteria is probably connected with reactions of the type



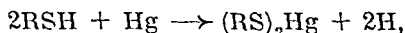
as e. g. the oxidation of cysteine



or glutathione



The reason why the mercury electrode cannot be used in this case is that mercury forms compounds with the sulphydryl group, partly of the type



partly more complicated complex salts, and the potential of the mercury electrode is determined by the equilibrium of these compounds. In the present investigation, therefore, mercury electrodes are avoided. In order to ascertain that the platinum electrodes gave constant potentials in solutions containing sulphydryl compounds, the potentials

of two cysteine solutions were measured, one acid solution and one alkaline (phosphate buffers). The following results were obtained:

Table 2.

Control of Platinum Electrode in Acid and Alkaline Cysteine Solution, showing that the Potentials in both Cases reach a Constant Level.

Time	Potential	
15 minutes	—0.4884 volt	—0.3432 volt
30 »	—0.4994 »	—0.3818 »
45 »	—0.5060 »	—0.4653 »
60 »	—0.5071 »	
75 »	—0.5071 »	
90 »	—0.5117 »	—0.4850 »
105 »	—0.5128 »	—0.4939 »
120 »	—0.5140 »	—0.4951 »
135 »	—0.5152 »	—0.4988 »
150 »	—0.5152 »	—0.4988 »
165 »	—0.5152 »	—0.4988 »

In both cases the potentials remain perfectly constant after 2½ hours.

A very important condition for constant potentials is the complete exclusion of oxygen. The drifting and irregular potentials observed by *Dixon* and *Quastel* with platinum electrodes are probably due to traces of oxygen. Great care therefore must be taken to exclude air during the experiment. Usually the exclusion of air is attained by leading a stream of nitrogen through the apparatus. For the long-continued experiments here involved, however, the usual laboratory gasometers do not contain sufficient quantities of gas, it is necessary to use nitrogen compressed in steel cylinders. The commercial nitrogen cylinders, however, always contain some oxygen, as the gas is prepared by fractional distillation of liquid air, and sufficient care has not been taken to secure complete separation of the oxygen. For the present experiments a cylinder of very pure argon was used instead of nitrogen, and this experimental improvement facilitated considerably the exclusion of the last traces of oxygen. The argon from the cylinder was purified by passage through a layer of hot copper wires and then through an alkaline pyrogallol solution. These operations were carried out by means of an apparatus which originally was constructed for another purpose, viz. the determination of sulphur and halogen in organic compounds by combustion in air (4) and absorption of the gaseous combustion products. The apparatus (Fig. 2) consists of a quartz tube CD where the combustion of the substance takes place, and an absorption apparatus DEFG. The container EF is filled with glass spherules in order to increase the contact surface between

the gas and the absorbent. In this container the greater part of the absorption takes place. In order to ensure a complete absorption of the last traces of absorbable gases, the gas mixture then passes a filter *F* with very narrow pores. Here the gas is distributed into minute bubbles which pass through a new layer of the absorbent *FG*.

In the present experiment, argon from the cylinder *A* was led into the apparatus through an india rubber tube *AB* and a glass

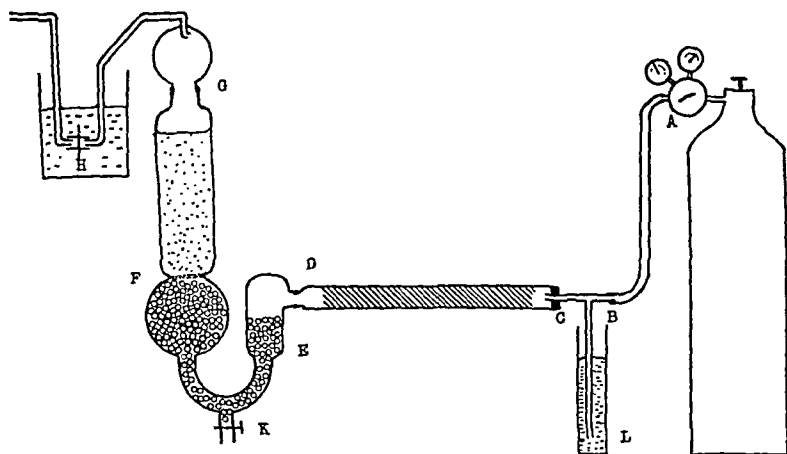


Fig. 2.

tube *BC*. In order to prevent too great pressures in the apparatus a mercury valve *L* is inserted. As soon as the pressure exceeds a certain value, the excess gas escapes through *L*. A penetration of gas from outside through *L* is impossible.

The glass tube *BC* is connected with the quartz tube *CD* through a rubber stopper. The tube *CD* is filled with freshly reduced copper wires and heated by means of a large burner. At *D* the tube is connected with the absorption apparatus through a well-ground glass stopper. The container *EFG* is filled with alkaline pyrogallol solution. At *G* the absorption apparatus is connected with a glass tube *GH* through a ground-in stopper. At *H* this tube is connected with a new tube leading into the electrode vessel containing the bacterial culture. This connection is made by means of a short vacuum rubber tube. The rate of the gas flow here can be adjusted by means of a pinchcock. In order to prevent diffusion of air from without, the rubber tube is placed in a beaker with water. As india rubber has an appreciable penetrability for gases, the use of rubber tubes is restricted as much as possible. The only place where the gas passes a rubber tube surrounded by air is the connection *AB*. Here, however, the gas has a considerable pressure which is necessary to force it through the filter *F*. The diffusion outwards therefore will be stronger than the diffusion inwards. If some oxygen should penetrate the rubber tube, it will be completely absorbed by passing the hot copper

wires and the alkaline pyrogallol solution. After passage through the absorption apparatus, the argon is not led through rubber tubes except at *H*, and here the admission of air is prevented by surrounding the tube with water.

In the absorption apparatus there are three connections with ground-in surfaces, viz. the stoppers *D* and *G* and the glass cock *K*. These surfaces are very carefully ground and properly lubricated.

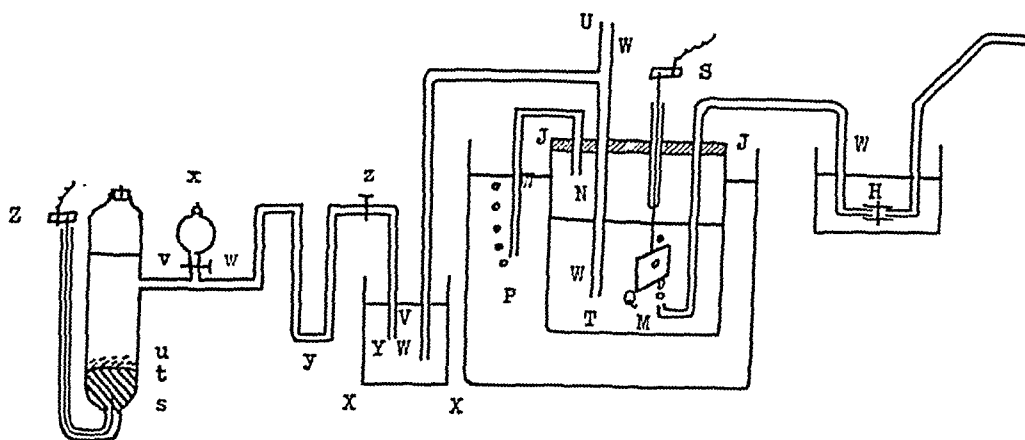


Fig. 3.

at *D* and *K* the gas pressure is considerable, and also at *G* the argon pressure exceeds the atmospheric pressure.

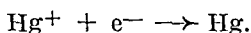
At two places rubber stoppers are used. The first place is at *C*. The argon here has a considerable pressure, and if any oxygen should enter at this place, it will be removed in the absorption apparatus. The second place where a rubber stopper is used is in the electrode vessel (*JJ* Fig. 3). Here also the argon pressure exceeds one atmosphere, and a continuous stream of argon passes the vessel. If any oxygen should leak through *JJ*, at least the greatest part will be transported out through the waste-pipe *NP* by the argon stream, and only a minimal quantity of oxygen will have any possibility of diffusing into the fluid in the vessel. The oxygen streaming out from *M* and surrounding the electrode *Q* at least will be completely pure, and if any trace of oxygen should diffuse through the liquid surface, it will be met by the ascending stream of argon. The excess of argon escapes through the waste-pipe *NP* which opens out under water in order to hinder the entrance of air.

In the electrode vessel is a platinum electrode *QS* and a h-shaped glass tube *TUV* filled with a 2 per cent agar solution saturated with potassium chloride. The inlet tube and the outlet tubes are closed with cotton plugs at the places designated *W* in fig. 3 in order to prevent infection of the bacterial culture in the vessel. The electrode vessel is placed in a water thermostat keeping the temperature at 37°.

The branch *UV* of the electrolyte bridge is immersed in a beaker *XX* containing potassium chloride solution, and in the same beaker the electrolyte bridge from the standard electrode ends.

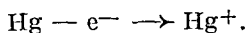
As was mentioned above, the normal hydrogen cell is used for theoretical electro-chemical studies. For experimental determinations, however, other standard cells are often preferred, and in the present investigation a decinormal calomel cell was used. This cell is contained in a glass vessel *ZswyY* of the shape seen in Fig. 3. A platinum wire *Zs* is introduced into a layer of mercury *st*. The mercury surface is covered with solid calomel *tu* and a layer of decinormal potassium chloride solution *uv* saturated with calomel. This solution also fills the tube *vyzY*. At *z* is a stop cock which is closed when the cell is not used for measurements, in order to protect the potassium chloride solution of the cell against contamination by diffusion from the beaker *V*. The S-form of the tube *vyzY* is also designed to impede diffusion. Potassium chloride solution saturated with calomel is re-filled in the container *xw*. Before the stopcock *z* is closed after a potential estimation, the electrolyte in the tube *wyz* is removed by opening the stopcock *w* for a moment to let fresh electrolyte solution from the container stream through the tube.

The electrochemically active components of this cell are mercury, calomel and decinormal potassium chloride solution. The cell can act as an oxidant, mercurous ions being reduced to atoms:



The mercurous ions are present in an aqueous potassium chloride solution saturated with calomel. The ions which are removed by the reduction are replaced by a corresponding quantity of solid calomel which is dissolved during the process, and the components of the electrolyte solution thus remain unaltered.

On the other hand, this cell can act as a reductant, mercury atoms being oxidised to mercurous ions:



The mercurous ions will enter the electrolyte solution, but as this solution is already saturated with calomel, a corresponding quantity of calomel will be precipitated, and the composition of the electrolyte solution also in this case remains constant. The mercurous ion concentration in the solution is constant, and depends only upon the potassium chloride concentration, as

$$[\text{Hg}^+] \cdot [\text{Cl}^-] = \text{constant} = \text{the solubility product of calomel},$$

and the chlorine ion concentration is determined by the potassium chloride solution.

The calomel cell was prepared immediately before the measure-

ments. Chemically pure, redistilled mercury, and calomel and potassium chloride preparations pro analysi were used. The calomel was shaken repeatedly with decinormal potassium chloride solution to remove traces of mercuric chloride. The potassium chloride was dried at 200°, cooled in desiccator, and dissolved in distilled water to deci-

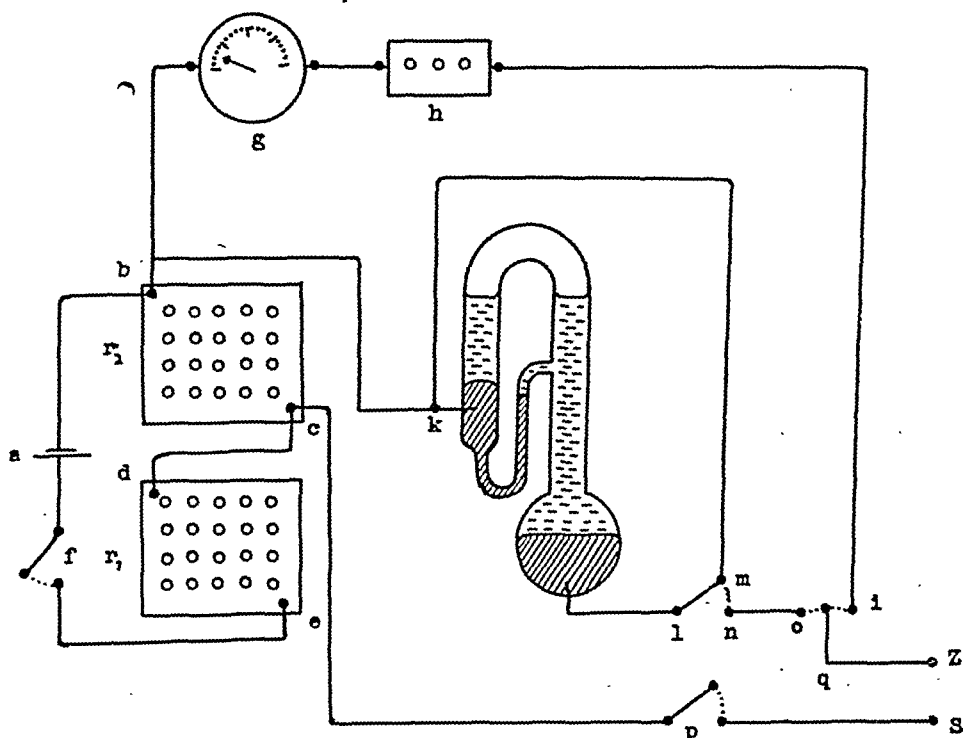


Fig. 4.

normal solution which was shaken with solid calomel for twenty minutes.

The potential was measured between the platinum electrode *S* in the bacterial culture and the electrode *Z* of the calomel cell. The estimations were made by the compensation method.

An accumulator *a* is connected with a circuit *abcdefa* through two variable precision resistances r_1 and r_2 . A switch *f* is introduced to break off the current in the intervals between the measurements. One of the accumulator poles is connected with the calomel cell *Z* through two circuits. One of these, *abghiZ* passes through an ampère-meter *g*, a variable resistance *h*, and a switch *iq*. This switch permits connection of the calomel cell with the accumulator alternating through the circuit already described, and the other circuit, *abklnoqZ* through a capillary electrometer *kl*. In this current is introduced a switch *lmn* to short-circuit the capillary electrometer when it is not used for measurements. The electrode *S* in the bacterial culture is con-

nected with the resistance r_2 at c . A switch p breaks the circuit when the apparatus is not used for measurements.

The complete apparatus is seen in Fig. 5.

The potential estimations are performed by adjusting the resistance r_2 until the ampère-meter and the galvanometer show absence of

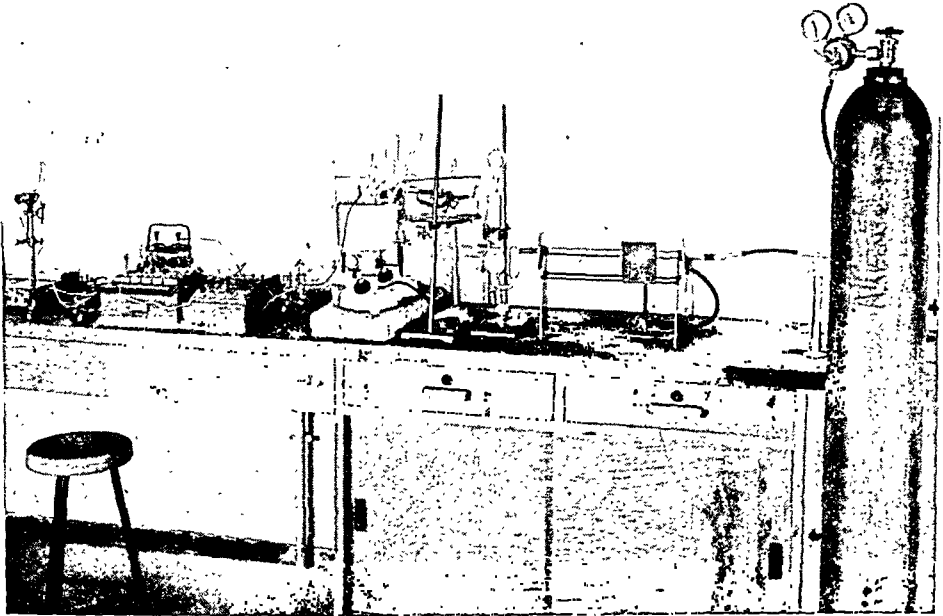


Fig. 5.

current in the circuits $SpcbghiqZ$ and $SpcbklnoqZ$ respectively. The switch f is turned to close the accumulator circuit $abcdefa$, and the switch oqi is turned to position qi . The electrode S is connected with c by means of the switch p . The resistance h has its maximum value. The ampère-meter g will then generally indicate a current in the circuit. The resistance r_2 is adjusted until the ampère-meter needle returns to zero. Then the resistance h is eliminated, and the resistance r_2 again adjusted to absence of current. Then the switch goi is turned to the position go , and resistance r_2 is adjusted until the capillary electrometer kl does not indicate current when the switch lnm is turned in position ln . Then the estimation is finished, and the circuits are broken with the switches f , p and oqi .

Let R_1 and R_2 be the resistance of r_1 and r_2 when the capillary electrometer indicates absence of current. Then the potential difference $E_{0.1c}$ between the electrodes S and Z is

$$E_{0.1c} = \frac{R_2}{R_1 + R_2} \cdot E_a,$$

E_a being the electromotive force of the accumulator. In the present experiments the electrode in the bacterial culture was combined with the negative pole of the accumulator, the calomel cell with the positive accumulator pole to obtain absence of current in the galvanometer circuit. The electrode in the culture then is negative compared with the decinormal calomel cell.

To determine the electromotive force of the accumulator, a standard cell is introduced instead of the electrodes Z and S . The cell used here was a Weston cell. The electromotive force of this cell is $1.01830 - 0.000038 (t - 20^\circ) - 0.00000065 (t - 20^\circ)^2$ volt, t being the temperature. The electromotive force of the standard cell being E_s , the electromotive force of the accumulator is

$$E_a = \frac{R_1 + R_2}{R_2} E_s,$$

Estimations made at 20° gave $R_1 = 10000$ ohm and $R_2 = 9507$ ohm, giving $E_a = 2.089$ volt. Control measurements showed that the electromotive force of the accumulator remained constant during the experiment.

Before the experiment, the electrode vessel with its inlet tube and outlet tubes (Fig. 3 from H to P and V) were carefully cleaned and dried. Then the tubes were closed with cotton plugs at the five places designated W , the electrode vessel was closed with the rubber stopper, and the vessel was sterilised in an autoclave for one hour at 120° .

Then the cotton plug at U was removed and the tube TUV was filled with a hot, sterile solution of 2 per cent agar in saturated potassium chloride solution, and the cotton plug was again placed into the tube. The agar solution was prepared by adding 2 gr. agar to 100 ml saturated potassium chloride solution and heating in an autoclave for one hour to 120° . Before the solution is filled into the electrolyte bridge TUV , it is heated to a thin fluid. On cooling it becomes gelatinous.

The rubber stopper JJ (Fig. 3) was removed for a moment, and the sterile medium was filled into the vessel and inoculated with the bacterium to be investigated. Then the vessel was closed, placed into the thermostat, and a stream of argon was led through the apparatus. (The bacteria were not cultivated in the usual culture tubes. The vessel containing the culture is a cylindrical glass beaker, diameter 3.5 cm., height 8 cm. 40 ml. of the medium was used for the experiment.) The growth of the culture is controlled by means of the light absorption. The purity of the strains is controlled by microscopical examination.

The potential estimations of the cysteine solutions (Table 2) showed that the potential is constant after $2\frac{1}{2}$ hours. The first potential estimation therefore was made 3 hours after the inoculation.

Two series of estimations were performed for each bacterium. The resistance R_1 was always 10000 ohm.

At first a series of *Clostridia* cultivated in Difco Bacto Brain Liver Heart medium was investigated. The medium is prepared by dissolving 46 grams of Difco Bacto Brain Liver Heart in 1000 ml. distilled water by boiling for a few minutes and sterilising in an autoclave at 120° for 30 minutes. The Difco Bacto Brain Liver Heart has the following composition:

Bacto-Liver, Infusion from	50	g.
Calf Brains, Infusion from	200	g.
Beef Heart, Infusion from	250	g.
Proteose-Peptone, Difco	10	g.
Neopeptone, Difco	3.25	g.
Bacto-Tryptone	3.25	g.
Bacto-Dextrose	2	g.
Sodium Chloride	5	g.
Disodium Phosphate	2.5	g.
Bacto-Agar	1.75	g.

Cultures of *Clostridium septicum* in Brain Liver Heart gave the following potentials:

Table 3.
Oxidation-reduction Potentials in Culture of *Cl.septicum*. Medium: Brain Liver Heart.

t in hours	R_2 in ohms	$E_{0.1c}$ in volt	E_h in volt	pH	$E_h + 0.0615 \text{ pH}$ in volt
a. Series 1.					
3	2274	—0.387	—0.050	6.33	0.339
5	2460	—0.413	—0.076	6.25	0.308
7	2526	—0.421	—0.084	6.16	0.295
10	3364	—0.526	—0.189	6.03	0.182
21	4083	—0.606	—0.269	5.70	0.082
34	3960	—0.593	—0.256	5.62	0.090
45	4096	—0.607	—0.270	5.63	0.076
53	4226	—0.621	—0.284	5.70	0.067
71	4200	—0.618	—0.281	5.93	0.084
81	4133	—0.611	—0.274	6.13	0.103
94	4120	—0.610	—0.273	6.37	0.119
b. Series 2.					
5	2507	—0.419	—0.082	6.25	0.302
7	2604	—0.432	—0.095	6.16	0.284
10	3387	—0.529	—0.192	6.03	0.179
21	4084	—0.606	—0.269	5.70	0.082
34	4000	—0.597	—0.260	5.62	0.086
45	4100	—0.608	—0.271	5.63	0.075
53	4239	—0.622	—0.285	5.70	0.066
71	4213	—0.619	—0.282	5.93	0.083
81	4204	—0.618	—0.281	6.13	0.096
94	4150	—0.613	—0.276	6.37	0.112

The potentials $E_{0.1c}$ referred to the decinormal calomel cell as standard are calculated from the resistances R_2 immediately determined by the experiment. The potentials E_h referred to the normal hydrogen electrode are given by the formula

$$E_h = E_{0.1c} + 0.337 \text{ volt.}$$

It is seen that the potentials E_h decrease rapidly during the first 24 hours, and then reach a rather constant level at -0.275 volt (Fig. 6).

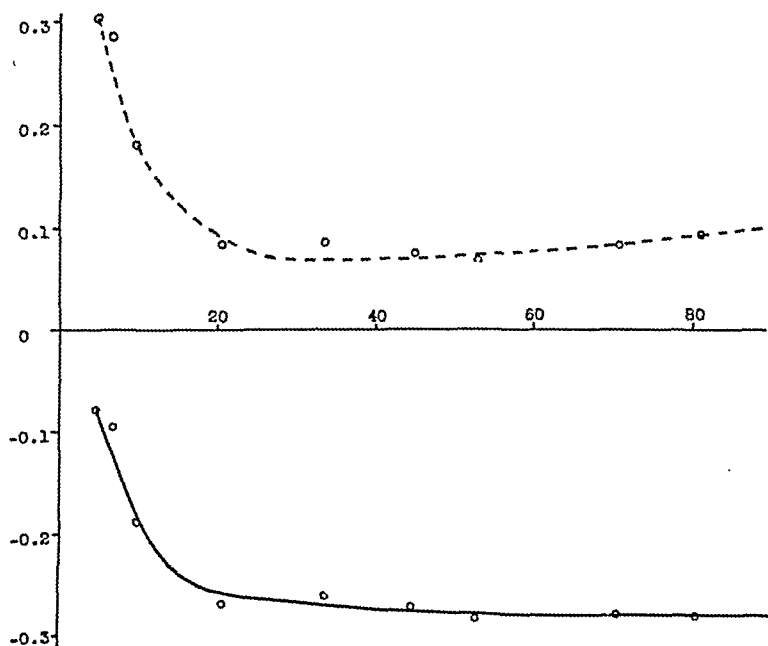
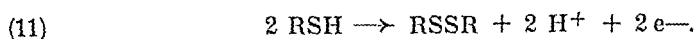


Fig. 6.
Cl. septicum. Abscissa: t in hours. Ordinate:
——— E_h in volt.
----- $E_h + 0.0615 \text{ pH}$ in volt.

It was mentioned above that oxidation of sulphydryl compounds plays an important part in cellular respiration. The oxidation is summarily expressed by the equation



Introducing this into equation (10) we obtain

$$\begin{aligned} E &= E_0 - 0.0001985 \frac{T}{2} \log \frac{[\text{RSH}]^2}{[\text{RSSR}] [\text{H}]^2} \\ &= E_0 + 0.0001985 T \log [\text{H}] - 0.0001985 T \log \frac{[\text{RSH}]}{\sqrt{[\text{RSSR}]}} \\ &= E_0 - 0.0001985 T \text{ pH} - 0.0001985 T \log \frac{[\text{RSH}]}{\sqrt{[\text{RSSR}]}}. \end{aligned}$$

Introducing the temperature of the present experiment, $T = 273 + 37^\circ$, the equation can be written

$$E = E_0 - 0.0615 \text{ pH} - 0.0615 \log \frac{[\text{RSH}]}{\sqrt{[\text{RSSR}]}}$$

The validity of this equation has been submitted to a series of experimental investigations. *Ghosh* et al. (5) found the equation to be valid for neutral and alkaline cysteine solutions. Acid cysteine solutions show irregular conditions, as the potentials are expressed by an equation of the form

$$E = E_0 - 0.0615 \text{ pH} - \text{const.} \log [\text{RSH}].$$

This result, already obtained by *Dixon* and *Quastel* (1), has been verified by a series of investigators. In both cases, however, the potential depends upon the hydrogen ion activity, and the dependence is expressed by a term -0.0615 pH . Of course it is not permissible to apply this equation to the complex reactions occurring in bacterial cultures, even if reactions of the form (11) play an important part in the metabolism of bacteria. The oxidation-reduction potentials, however, will certainly depend upon the hydrogen ion activity, and the expression $E_h + 0.0615 \text{ pH}$ is calculated in the tables. For reactions of the form (11) this expression represents the terms

$$E_0 - 0.0615 \log \frac{[\text{RSH}]}{\sqrt{[\text{RSSR}]}}$$

The hydrogen ion determinations were made in separate cultures. A glass electrode was used in connection with an ionometer (Rørdionometer Type PHM 11, Radiometer, Copenhagen). The pH-values of Table 3 are determined by interpolation in the following series of observations:

Table 4.
Variation of Hydrogen Ion Activity in Culture of *Cl.septicum*. Medium: Brain Liver Heart.

t in hours	pH observed	pH graphically smoothed	d pH dt
0	6.45	6.45	-0.042
2	6.38	6.37	-0.042
4	6.28	6.29	-0.042
6	6.30	6.21	-0.042
8	6.20	6.12	-0.041
10	6.03	6.03	-0.040
12	5.93	5.95	-0.038
14	5.79	5.87	-0.035
25	5.63	5.65	-0.012
33	5.66	5.62	-0.003
38	5.62	5.62	0.001
49	5.68	5.67	0.008
73	5.98	5.98	0.020
86	6.23	6.23	0.020

The hydrogen ion concentration increases during the first 24 hours, in the growth phase of the bacteria. Then it remains rather constant for the next 24 hours, and then it decreases slowly as the autolysis of dead bacteria sets in.

The expression $E_h + 0.0615 \text{ pH}$ decreases during the first 24 hours, remains constant for the next 24 hours, to show a slight increase towards the end of the experiment.

It is seen that the accuracy of measurement is considerably less in these complicated systems of bacterial cultures than in the pure cysteine solution (Table 2).

The next bacterium to be investigated was *Clostridium tetani*.

Table 5.

Oxidation-reduction Potentials in Culture of *Cl.tetani*. Medium: Brain Liver Heart.

t in hours	R ₂ in ohm	E _{o.1c} in volt	E _h in volt	pH	E _h + 0.0615 pH in volt
a. Series 1.					
3	2274	—0.387	—0.050	6.34	0.340
5	3174	—0.503	—0.166	6.26	0.219
7	3857	—0.582	—0.245	6.19	0.136
10	4189	—0.617	—0.280	6.09	0.095
21	4374	—0.636	—0.299	6.06	0.074
27	4360	—0.634	—0.297	6.15	0.081
34	4273	—0.626	—0.288	6.28	0.098
45	4175	—0.615	—0.278	6.58	0.127
53	4325	—0.631	—0.294	6.81	0.125
71	4284	—0.627	—0.290	7.14	0.149
81	4223	—0.620	—0.283	7.14	0.156
94	4257	—0.624	—0.287	7.14	0.152
b. Series 2.					
5	3334	—0.522	—0.185	6.26	0.200
7	4040	—0.597	—0.260	6.19	0.121
10	4197	—0.617	—0.280	6.09	0.095
21	4377	—0.636	—0.299	6.06	0.074
27	4373	—0.636	—0.299	6.15	0.079
34	4277	—0.626	—0.289	6.28	0.097
45	4174	—0.615	—0.278	6.58	0.127
53	4335	—0.632	—0.295	6.81	0.124
71	4300	—0.628	—0.291	7.14	0.148
81	4285	—0.627	—0.290	7.14	0.149
94	4266	—0.625	—0.288	7.14	0.151

The hydrogen ion activities were determined by interpolation in the following table of observations:

Table 6.

Variation of Hydrogen Ion Activity in Culture of *Cl.tetani*. Medium: Brain Liver Heart.

t in hours	pH observed	pH graphically smoothed	$\frac{d \text{ pH}}{dt}$
0	6.45	6.45	— 0.036
2	6.38	6.38	— 0.036
4	6.08	6.30	— 0.036
6	6.35	6.23	— 0.036
8	6.33	6.16	— 0.036
10	6.10	6.09	— 0.036
12	6.10	6.04	— 0.022
14	5.93	6.02	— 0.005
25	6.17	6.12	0.018
33	6.26	6.26	0.024
38	6.38	6.39	0.030
49	6.74	6.72	0.030
62	7.11	7.02	0.017
73	7.17	7.14	0.008
86	7.07	7.14	0.000
98	7.13	7.14	0.000

The potentials found for *Cl. tetani* resemble those of *Cl. septicum*. The only difference is that they decrease somewhat more rapidly, and reach a lower constant level at $E_h = -0.29$ volt.

Clostridium perfringens cultivated in the same medium gave the following potentials:

Table 7.

Oxidation-reduction Potentials in Culture of *Cl.perfringens*. Medium: Brain Liver Heart.

t in hours	R ₂ in ohm	E _{0,1c} in volt	E _h in volt	pH	E _h + 0.0615 pH in volt
a. Series 1.					
3	2270	— 0.387	— 0.050	6.26	0.335
5	2497	— 0.418	— 0.081	6.14	0.297
7	2670	— 0.440	— 0.103	6.03	0.268
10	3073	— 0.491	— 0.154	5.88	0.208
21	3743	— 0.569	— 0.232	5.84	0.127
27	3842	— 0.580	— 0.243	5.92	0.121
34	3930	— 0.590	— 0.253	6.02	0.117
45	4100	— 0.608	— 0.271	6.21	0.111
53	4174	— 0.615	— 0.278	6.40	0.116
71	4349	— 0.633	— 0.296	6.74	0.119
81	4420	— 0.640	— 0.303	6.83	0.117
94	4570	— 0.656	— 0.319	6.87	0.104

t	R ₂	E _{0.1c}	E _h	pH	E _h + 0.0615 pH
b. Series 2.					
5	2507	—0.419	—0.082	6.14	0.296
7	2690	—0.443	—0.106	6.03	0.265
10	3084	—0.493	—0.156	5.88	0.206
21	3765	—0.572	—0.235	5.84	0.124
27	3844	—0.580	—0.243	5.92	0.121
34	3933	—0.590	—0.253	6.02	0.117
45	4100	—0.608	—0.271	6.21	0.111
53	4184	—0.616	—0.279	6.40	0.115
71	4370	—0.635	—0.298	6.74	0.117
81	4425	—0.641	—0.304	6.83	0.116
94	4660	—0.664	—0.327	6.87	0.096

The potentials reach a constant level at $E_h = -0.30$ volt. The hydrogen ion activity observations show the usual result: a decrease during the multiplication phase and then an increase:

Table 8.
Variation of Hydrogen Ion Activity in Culture of *Cl. perfringens*. Medium:
Brain Liver Heart.

t in hours	pH observed	pH graphically smoothed	$\frac{d \text{ pH}}{dt}$
0	6.45	6.45	—0.061
2	6.27	6.32	—0.061
4	6.08	6.20	—0.060
6	6.35	6.08	—0.058
8	6.00	5.97	—0.054
10	5.84	5.88	—0.043
12	5.78	5.78	—0.035
14	5.64	5.73	—0.009
25	5.89	5.88	0.014
33	5.96	6.00	0.016
38	6.05	6.09	0.016
49	6.29	6.29	0.023
62	6.67	6.60	0.019
73	6.82	6.77	0.009
86	6.77	6.85	0.001
98	6.87	6.87	0.000

In cultures of *Clostridium sporogenes* in Brain Liver Heart medium the following potentials were found:

Table 9.

Oxidation-reduction Potentials in Culture of *Cl.sporogenes*. Medium: Brain Liver Heart.

t in hours	R ₂ in ohm	E _{0,1c} in volt	E _h in volt	pH	E _h + 0.0615 pH in volt
a. Series 1.					
3	2274	—0.387	—0.050	6.37	0.342
5	3353	—0.525	—0.188	6.32	0.201
7	4003	—0.597	—0.260	6.29	0.127
10	4216	—0.620	—0.283	6.26	0.102
27	4214	—0.620	—0.283	6.47	0.115
34	4407	—0.639	—0.302	6.57	0.102
45	4573	—0.656	—0.319	6.75	0.096
53	4576	—0.656	—0.319	6.87	1.004
71	4614	—0.660	—0.323	7.03	0.109
81	4692	—0.667	—0.330	7.08	0.105
94	4603	—0.659	—0.322	7.12	0.116
b. Series 2.					
5	3404	—0.531	—0.194	6.32	0.195
7	4144	—0.612	—0.275	6.29	0.112
10	4242	—0.622	—0.285	6.26	0.100
27	4223	—0.620	—0.283	6.47	0.115
34	4403	—0.639	—0.302	6.57	0.102
45	4585	—0.657	—0.320	6.75	0.095
53	4580	—0.656	—0.319	6.87	0.104
71	4624	—0.661	—0.324	7.03	0.108
81	4696	—0.668	—0.331	7.08	0.104
94	4664	—0.665	—0.328	7.12	0.110

The potentials decrease to the level $E_h = -0.325$ volt. The pH determinations gave the following results:

Table 10.

Variation of Hydrogen Ion Activity in Culture of *Cl.sporogenes*. Medium: Brain Liver Heart.

t in hours	pH observed	pH graphically smoothed	$\frac{d \text{ pH}}{dt}$
0	6.45	6.45	—0.033
2	6.40	6.39	—0.027
4	6.24	6.34	—0.017
6	6.23	6.30	—0.014
8	6.35	6.28	—0.011
10	6.32	6.26	—0.008
12	6.25	6.25	0.000
14	6.25	6.26	0.005
25	6.50	6.44	0.017
33	6.50	6.55	0.016
38	6.49	6.63	0.016
62	6.96	6.97	0.010
73	7.13	7.05	0.005
86	7.02	7.10	0.000
98	7.12	7.12	0.000

Cl. tetanomorphum, cultivated in Brain Liver Heart medium, showed the following potentials:

Table 11. Oxidation-reduction Potentials in Culture of *Cl. tetanomorphum*.
Medium: Brain Liver Heart.

t in hours	R ₂ in ohm	E _{0,1c} in volt	E _h in volt	pH	E _h + 0.0615 pH in volt
3	2270	—0.387	—0.050	6.41	0.344
5	2634	—0.436	—0.099	6.39	0.294
7	3177	—0.504	—0.167	6.37	0.225
10	3732	—0.568	—0.231	6.34	0.159
21	4130	—0.611	—0.274	5.90	0.089
27	4214	—0.619	—0.282	5.60	0.062
34	4319	—0.630	—0.293	5.58	0.050
45	4429	—0.641	—0.304	5.71	0.047
53	4487	—0.647	—0.310	5.89	0.052
71	4397	—0.638	—0.301	6.14	0.077
81	4346	—0.633	—0.296	6.15	0.082
94	4302	—0.629	—0.292	6.17	0.087

b. Series 2.

5	2660	—0.439	—0.102	6.39	0.291
7	3190	—0.505	—0.168	6.37	0.224
10	3740	—0.569	—0.232	6.34	0.158
27	4244	—0.623	—0.291	5.60	0.053
34	4360	—0.634	—0.297	5.58	0.046
45	4444	—0.643	—0.306	5.71	0.045
53	4494	—0.648	—0.311	5.89	0.051
71	4432	—0.642	—0.305	6.14	0.073
81	4352	—0.634	—0.297	6.15	0.081
94	4343	—0.633	—0.296	6.17	0.083

The potentials decrease to the constant level $E_h = -0.305$ volt.

Table 12. Variation of Hydrogen Ion Activity in Culture of *Cl. tetanomorphum*.
Medium: Brain Liver Heart.

t in hours	pH observed	pH graphically smoothed	$\frac{d \text{ pH}}{dt}$
0	6.45	6.45	—0.010
2	6.40	6.42	—0.010
4	6.29	6.40	—0.010
6	6.35	6.38	—0.010
8	6.37	6.36	—0.010
10	6.35	6.34	—0.010
12	6.35	6.32	—0.012
14	6.31	6.29	—0.029
25	5.64	5.64	—0.031
33	5.58	5.58	0.000
38	5.62	5.61	0.008
49	5.77	5.79	0.024
62	6.11	6.10	0.011
73	6.15	6.14	0.001
86	6.12	6.16	0.000
98	6.21	6.18	0.000

Culture of *Cl. centrosporogenes* in Brain Liver Heart medium gave the potentials:

Table 13.
Oxidation-reduction Potentials in Culture of *Cl. centrosporogenes*.
Medium: Brain Liver Heart.

t in hours	R ₂ in ohm	E _{0.1c} in volt	E _h in volt	pH	E _h + 0.0615 pH in volt
a. Series 1.					
4	2093	— 0.362	— 0.025	6.28	0.361
16	3603	— 0.553	— 0.216	5.61	0.129
23	3837	— 0.579	— 0.242	5.73	0.110
29	4003	— 0.597	— 0.260	5.83	0.099
40	4116	— 0.609	— 0.272	6.03	0.099
48	4205	— 0.619	— 0.282	6.18	0.098
64	4231	— 0.621	— 0.284	6.47	0.114
b. Series 2.					
4	2144	— 0.369	— 0.032	6.28	0.354
16	3657	— 0.560	— 0.223	5.61	0.122
23	3876	— 0.584	— 0.252	5.73	0.100
29	3997	— 0.597	— 0.260	5.83	0.099
40	4142	— 0.612	— 0.275	6.03	0.096
48	4242	— 0.622	— 0.285	6.18	0.095
64	4252	— 0.623	— 0.286	6.47	0.112

The potentials observed in this strain decrease to E_h = — 0.28 volt, and do not reach a so distinctly constant level as the preceeding strains.

Table 14.
Variation of Hydrogen Ion Activity in Culture of *Cl. centrosporogenes*.
Medium: Brain Liver Heart.

t in hours	pH observed	pH graphically smoothed	$\frac{d \text{ pH}}{dt}$
0	6.45	6.45	— 0.004
2	6.41	6.41	— 0.045
4	6.27	6.28	— 0.081
6	6.12	6.08	— 0.099
8	5.86	5.86	— 0.100
10	5.68	5.68	— 0.067
12	5.57	5.57	— 0.023
14	5.59	5.58	0.015
33	5.91	5.91	0.017
38	5.93	6.00	0.017
49	6.20	6.20	0.017
62	6.48	6.45	0.013
73	6.61	6.55	0.006
86	6.53	6.63	0.005
98	6.76	6.71	0.005

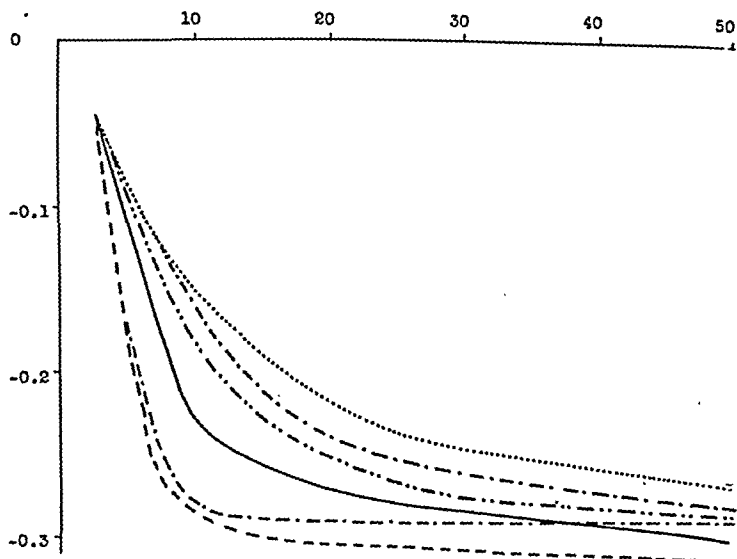


Fig. 7.

Abscissa: t in hours. Ordinate: E_h in volt.

- Cl. perfringens.
- . - . - . Cl. centrosporogenes.
- Cl. septicum.
- Cl. tetanomorphum.
- - - - - Cl. tetani.
- - - - - Cl. sporogenes.

A comparison of the *Clostridia* shows a striking similarity. The potentials decrease during the first 24 hours, and reach a rather constant level at -0.275 to -0.325 volt. The bacteria as yet considered are obligatory anaerobes. Afterwards strains of facultative anaerobes were investigated. Also these bacteria were cultivated in Brain Liver Heart Medium under anaerobic conditions. The first strain was *Staphylococcus aureus*:

Table 15.

Oxidation-reduction Potentials in culture of *Staphylococcus aureus* cultivated anaerobically in Brain Liver Heart Medium.

t in hours	R_2 in ohm	$E_{0.1c}$ in volt	E_h in volt	pH	$E_h + 0.0615 \text{ pH}$ in volt
a. Series 1.					
3	1977	-0.345	-0.008	6.35	0.383
4	2220	-0.380	-0.043	6.30	0.344
5	2240	-0.382	-0.045	6.18	0.335
6	2273	-0.387	-0.050	6.00	0.319
7	2300	-0.391	-0.054	5.70	0.297
12	2290	-0.389	-0.052	5.30	0.274
24	2366	-0.400	-0.063	5.30	0.263
36	2590	-0.430	-0.093	5.31	0.234
48	2603	-0.432	-0.095	5.42	0.238
72	2870	-0.466	-0.129	5.94	0.236

t	R ₂	E _{0.1c}	E _h	pH	E _h + 0.0615 pH
b. Series 2.					
3	1980	—0.345	—0.008	6.35	0.383
4	2233	—0.381	—0.044	6.30	0.343
5	2300	—0.391	—0.054	6.18	0.326
6	2290	—0.389	—0.052	6.00	0.317
7	2323	—0.394	—0.057	5.70	0.294
12	2245	—0.383	—0.046	5.30	0.280
24	2433	—0.409	—0.072	5.30	0.254
36	2672	—0.441	—0.104	5.31	0.223
48	2709	—0.442	—0.105	5.42	0.226
72	2899	—0.470	—0.133	5.94	0.232

Here the potentials differ remarkably from those found in cultures of the *Clostridia*. They decrease much more slowly, and do not by far reach so large negative values as those of the *Clostridia*. The potentials of the *Clostridia* decline very rapidly during the first 12 hours, and after 24 hours they are rather constant. The potentials of *Staphylococcus aureus* show a more uniform decline during the first 48 hours.

Table 16.

Variation of Hydrogen Ion Activity in Culture of *Staphylococcus aureus*.
Medium: Brain Liver Heart.

t in hours	pH observed	pH graphically smoothed	$\frac{d \text{ pH.}}{dt}$
0	6.45	6.45	—0.023
2	6.39	6.39	—0.036
4	6.30	6.30	—0.074
6	6.00	6.00	—0.200
8	5.47	5.47	—0.085
10	5.35	5.35	—0.038
12	5.30	5.30	—0.013
14	5.30	5.30	—0.001
25	5.39	5.30	0.000
33	5.36	5.30	0.000
38	5.28	5.31	0.002
49	5.37	5.43	0.020
62	5.79	5.74	0.020
73	6.03	5.96	0.018
86	6.07	6.13	0.010
98	6.24	6.23	0.005

Escherichia coli cultivated under the same conditions gave the following potentials:

Table 17.
Oxidation-reduction Potentials in Culture of *Escherichia coli*.
Medium: Brain Liver Heart. Anaerobically cultivated.

t in hours	R ₂ in ohm	E _{0.1c} in volt	E _h in volt	pH	E _h + 0.0615 pH in volt
a. Series 1.					
4	2000	-0.348	-0.011	6.27	0.375
23	2707	-0.445	-0.108	6.03	0.263
29	2907	-0.471	-0.134	6.10	0.241
40	3112	-0.496	-0.159	6.31	0.229
48	3205	-0.507	-0.170	6.50	0.230
64	3300	-0.518	-0.181	7.08	0.254
b. Series 2.					
4	2034	-0.353	-0.016	6.27	0.370
16	2532	-0.422	-0.085	6.00	0.286
23	2723	-0.447	-0.110	6.03	0.265
29	2912	-0.471	-0.134	6.10	0.254
40	3232	-0.510	-0.173	6.31	0.227
48	3246	-0.512	-0.175	6.50	0.225
64	3400	-0.530	-0.193	7.08	0.242

The potentials found in culture of *Escherichia coli* resemble the potentials of *Staphylococcus aureus*, but they reach more negative

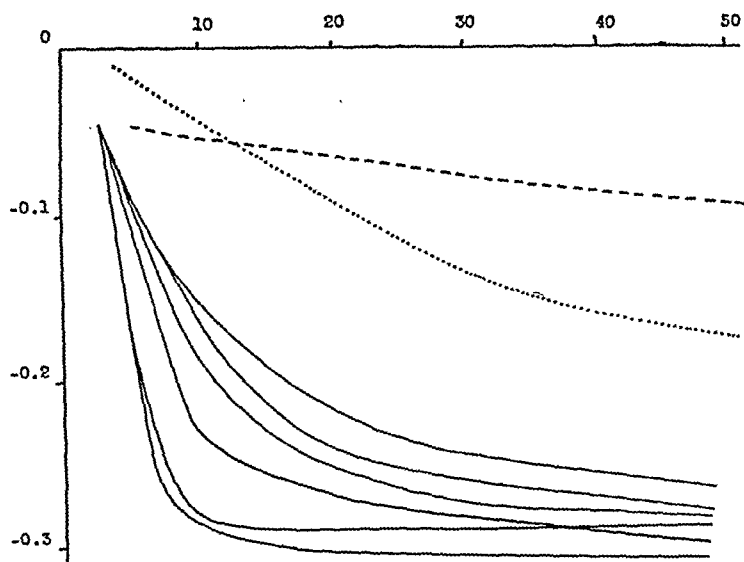


Fig. 8.

— Clostridia.
- - - Staphylococcus aureus.
..... Escherichia coli.
Abscissa: t in hours. Ordinate: E_h in volt.
Medium: Brain Liver Heart.

values. The potential curve differs decidedly from the curves of the *Clostridia* (Fig. 8).

Table 18.
Variation of Hydrogen Ion Activity in Culture of *Escherichia coli*.
Medium: Brain Liver Heart.

t in hours	pH observed	pH graphically smoothed	$\frac{d \text{ pH}}{dt}$
0	6.45	6.45	—0.063
2	6.35	6.36	—0.045
4	6.31	6.27	—0.037
25	6.07	6.05	0.005
33	6.17	6.17	0.018
49	6.53	6.53	0.026
62	7.04	7.00	0.040
73	7.66	7.44	0.040
86	7.87	7.96	0.040
98	8.38	8.38	0.040

The experiments show a marked difference between the obligatory and the facultative anaerobes investigated. The potential curves of the obligatory anaerobic *Clostridia* show a rapid decline during the first 12 hours after the inoculation, then the potential decreases more slowly for 24 hours, and after 36 hours the potential remains rather constant at a strongly negative level. The facultative anaerobes produced a more slow and uniform potential decline, and no distinctly constant level was reached. The potentials in these cultures are considerably more positive than the potentials of the obligatory anaerobes:

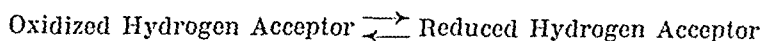
	Potential after 60 hours:
<i>Cl. sporogenes</i>	—0.32 volt
<i>Cl. tetanomorphum</i>	—0.30 -
<i>Cl. tetani</i>	—0.29 -
<i>Cl. perfringens</i>	—0.28 -
<i>Cl. septicum</i>	—0.28 -
<i>Cl. centrosporogenes</i>	—0.28 -
<i>Escherichia coli</i>	—0.18 -
<i>Staphylococcus aureus</i>	—0.11 -

In either case, however, the oxidation-reduction potential decreases during the growth of the culture. A displacement of the oxydation-reduction potential in a negative direction means that the culture becomes more reducing. As the bacterial respiration is con-

nected with oxidations, this result is surprising. It might be expected to observe an increase of the potential as the products of the oxidation are accumulated and simultaneously the concentration of the initial reduced substances is diminished, and the oxidation-reduction potential is determined by the formula

$$E = E_0 - 0.0001985 \frac{T}{n} \log \frac{[\text{Reductant}]}{[\text{Oxidant}]}$$

The observed effect may be explained as follows: The oxidation in absence of oxygen is realized by means of hydrogen acceptors which, as a consequence, are concentrated in the reduced form at the sacrifice of the substances which are oxidised by the bacterial respiration. While the oxidation is irreversible and the substances formed are only slightly electrochemically active, the reaction

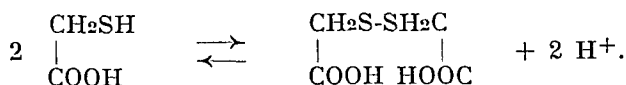


is reversible, and the oxidation-reduction potential is mainly determined by this equilibrium. During the growth of the bacteria, this equilibrium is displaced towards the reduced form, and the oxidation-reduction potential consequently decreases.

The reducing activity of bacteria has been demonstrated by means of methylene blue. *McLeod* and *Gordon* (6) investigated the capacity of various bacteria to reduce oxidised glutathione, studying their capacity to produce a positive nitroprusside reaction in meat extract and yeast extract which had been exposed to the air till the original nitroprusside reaction had disappeared, and could demonstrate a more definite gradation of their reducing activities than could be obtained with methylene blue. The bacteria investigated fell into four classes: 1. weak reducers: *Streptococci* and *Shigella dysenteriae*; 2. moderately active reducers: *Staphylococcus* and *Pneumococcus*; 3. active reducers: the coliform bacteria; 4. very active reducers: the obligatory anaerobes. Evidence for a roughly similar gradation of bacterial activity in reducing cystine to cysteine was obtained by *McLeod* and *Gordon* (7) in the production of haloes of clearing around colonies on an agar medium rendered opaque with excess of undissolved cystine. Since cystine is relatively insoluble and cysteine rather soluble, this result would be expected to follow reduction. It is seen that the gradation obtained by *McLeod* and *Gordon* is confirmed by the present oxidation-reduction measurements.

The next experiment is intended to determine whether it is possible to eliminate the sudden fall of the oxidation-reduction potential by adding a substance which stabilises the potential. For this purpose *Cl. sporogenes* was cultivated in a Brain Liver Heart medium to which

was added 0.1 per cent of thioglycolic acid.*) This acid can be reversibly oxidised:



The corresponding oxidation-reduction potential accordingly is

$$E = E_0 - 0.0001985.T.pH - 0.0001985.T.\log \frac{[\text{C}_2\text{O}_2\text{H}_4\text{S}]}{\sqrt{[\text{C}_4\text{O}_4\text{H}_6\text{S}_2]}}$$

The following potentials were found:

Table 19.

Oxidation-reduction Potentials in Culture of *Cl. sporogenes*.
Medium: Brain Liver Heart with 1 per cent Thioglycolic Acid.

t in hours	R ₂ in ohm	E _{0.1c} in volt	E _h in volt	pH	E _h + 0.0615 pH in volt
a. Series 1.					
4	2004	—0.349	—0.012	5.49	0.326
16	2107	—0.364	—0.027	5.38	0.304
23	2125	—0.366	—0.029	5.35	0.300
29	2136	—0.368	—0.031	5.36	0.299
40	2140	—0.368	—0.031	5.45	0.304
48	2195	—0.376	—0.039	5.60	0.305
64	2152	—0.370	—0.033	5.93	0.332
b. Series 2.					
4	2023	—0.352	—0.015	5.49	0.323
16	2143	—0.369	—0.032	5.38	0.299
23	2137	—0.368	—0.031	5.35	0.298
29	2163	—0.372	—0.035	5.36	0.295
40	2152	—0.370	—0.033	5.45	0.302
48	2200	—0.377	—0.040	5.60	0.304
64	2163	—0.372	—0.035	5.93	0.331

The effect is very striking. In this medium the potential only shows a slight decline and soon reaches a constant level. The potentials found in this experiment are much more positive than those found when the bacterium was cultivated in the pure Brain Liver Heart medium.

*) The addition of thioglycolic acid in order to stabilise the oxidation-reduction potential has been proposed by *Brewer* (8). *Brewer's* medium is used in the present laboratory and according to our experience it is well suited for the cultivation of anaerobic bacteria.

Table 20.

Variation of Hydrogen Ion Activity in Culture of *Cl. sporogenes* in Brain Liver Heart Medium with 0.1 per cent Thioglycolic Acid:

t in hours	pH observed	pH graphically smoothed	$\frac{d \text{ pH}}{dt}$
0	5.77	5.77	—0.150
2	5.54	5.55	—0.099
4	5.48	5.49	—0.021
6	5.48	5.46	—0.011
8	5.44	5.44	—0.008
10	5.43	5.42	—0.007
12	5.40	5.40	—0.006
14	5.40	5.39	—0.005
25	5.35	5.35	0.000
33	5.40	5.38	0.006
38	5.43	5.43	0.013
49	5.63	5.63	0.023
62	5.92	5.90	0.019
73	6.00	5.98	0.002
86	5.94	5.99	0.001
98	6.02	6.00	0.000

The influence of the medium upon the oxidation-reduction potential is further studied by cultivating the same bacterium (*Cl. sporogenes*) in a potato broth. This medium is prepared by adding a piece of sterile raw potato to a broth of the usual type.

The potential estimations in this medium gave the following results:

Table 21.

Oxidation-reduction Potentials in Culture of *Cl. sporogenes*.
Medium: Potato Broth.

t in hours	R ₂ in ohm	E _{0.1c} in volt	E _h in volt	pH	E _h + 0.0615 pH in volt
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a. Series 1.

5	2337	—0.396	—0.059	7.31	0.391
16	3155	—0.501	—0.164	7.03	0.268
24	2944	—0.475	—0.138	6.96	0.290
29	3110	—0.496	—0.159	6.93	0.266
40	3433	—0.534	—0.197	7.03	0.235
65	3370	—0.527	—0.190	7.74	0.286
89	3080	—0.492	—0.155	7.99	0.336

t	R ₂	E _{0.1c}	E _h	pH	E _h + 0.0615 pH
b. Series 2.					
5	2430	—0.409	—0.072	7.31	0.378
16	3162	—0.502	—0.165	7.03	0.267
24	3040	—0.487	—0.150	6.96	0.278
29	3120	—0.497	—0.160	6.93	0.265
40	3477	—0.539	—0.202	7.03	0.230
65	3474	—0.539	—0.202	7.74	0.274

The medium too has some stabilising effect upon the oxidation-reduction potential, but not by far so pronounced as the thioglycolic medium. In potato broth the potential falls rather rapidly during the first 24 hours, then more slowly, and after 48 hours a constant level at —0.20 volt is reached. In Brain Liver Heart medium the constant level was as —0.325 volt, in the thioglycolic medium —0.030 volt. As to the variation of the oxidation-reduction potential the potato broth thus holds an intermediate position between the Brain Liver Heart medium and the thioglycolic medium, but resembles more the former than the latter (Fig. 9).

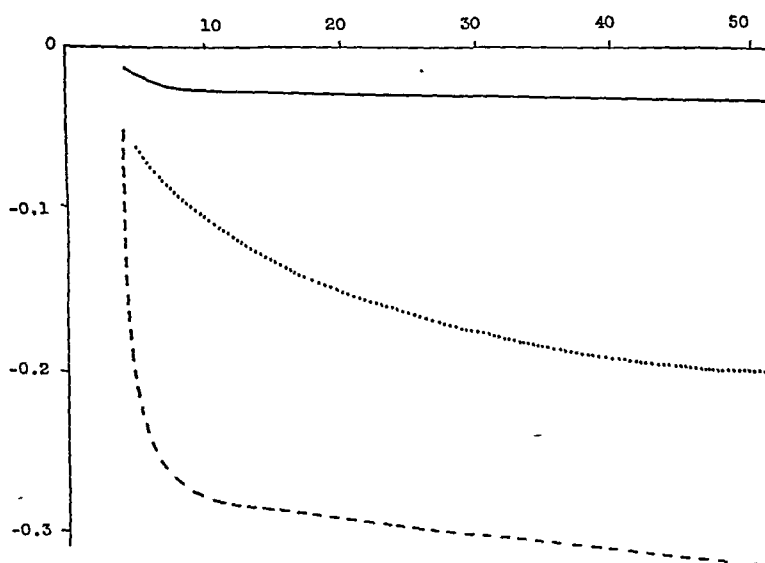


Fig. 9.
Cl. sporogenes.
Abscissa: t in hours. Ordinate: E_h in volt.
——— Thioglycolic Acid Medium.
..... Potato Broth.
- - - - Brain Liver Heart.

Table 22.
Variation of Hydrogen Ion Activity in Culture of *Cl. sporogenes*.
Medium: Potato Broth.

t in hours	pH observed	pH graphically smoothed	$\frac{d \text{ pH}}{dt}$
0	7.50	7.50	—0.098
2	7.25	7.34	—0.053
4	7.18	7.25	—0.035
6	7.17	7.19	—0.025
8	7.19	7.14	—0.018
10	7.17	7.11	—0.013
12	7.16	7.09	—0.012
14	7.10	7.06	—0.011
25	6.90	6.95	—0.007
33	7.00	6.95	0.004
38	7.00	7.01	0.015
49	7.19	7.22	0.030
62	7.70	7.67	0.029
73	7.87	7.87	0.013
98	8.08	8.08	0.001

A strain of *Cl. multifementans* cultivated in potato broth gave the following potentials:

Table 23.
Oxidation-reduction Potentials in Culture of *Cl. multifementans*
in Potato Broth.

t in hours	R ₂ in ohm	E _{0.1c} in volt	E _h in volt	pH	E _h + 0.0615 pH in volt
a. Series 1.					
5	2167	—0.372	—0.035	7.15	0.405
24	3560	—0.549	—0.212	7.05	0.222
29	3784	—0.574	—0.237	7.02	0.195
40	4100	—0.608	—0.271	6.96	0.157
65	4693	—0.667	—0.330	7.15	0.110
89	5070	—0.703	—0.366	7.25	0.080
b. Series 2.					
5	2333	—0.395	—0.058	7.15	0.382
24	3590	—0.552	—0.215	7.05	0.219
29	3814	—0.577	—0.240	7.02	0.192
40	4133	—0.611	—0.274	6.96	0.154
65	4737	—0.672	—0.335	7.15	0.105
89	5114	—0.707	—0.370	7.25	0.076

In contrast to the bacteria so far considered, *Cl. multifementans* do not give constant potentials in the course of the experiment, and the potentials reached by this bacterium are more negative than any of the potentials produced by the preceding bacteria.

Table 24.

Variation of Hydrogen Ion Activity in Culture of *Cl. multifementans* in Potato Broth.

t in hours	pH observed	pH graphically smoothed	$\frac{d \text{ pH}}{dt}$
0	7.50	7.50	—0.236
2	7.19	7.22	—0.055
4	7.15	7.16	—0.015
6	7.13	7.14	—0.008
8	7.13	7.13	—0.007
10	7.13	7.12	—0.006
12	7.12	7.10	—0.005
14	7.09	7.09	—0.005
25	7.09	7.05	—0.005
33	6.98	7.00	—0.005
38	6.93	6.93	—0.004
49	6.97	6.97	0.011
62	7.13	7.13	0.009
73	7.19	7.19	0.007
86	7.09	7.24	0.007
98	7.28	7.27	0.007

A strain of *Actinomyces necrophorus* in potato broth gave the following potentials:

Table 25.

Oxidation-reduction Potentials in Culture of *Actinomyces necrophorus* in Potato Broth.

t in hours	R ₂ in ohm	E _{0.1c} in volt	E _h in volt	pH	E _h + 0.0615 pH in volt
a. Series 1.					
5	2660	—0.439	—0.102	7.10	0.335
16	3852	—0.581	—0.244	6.73	0.170
24	4109	—0.609	—0.272	6.61	0.135
29	4111	—0.609	—0.272	6.65	0.137
40	4070	—0.604	—0.267	6.90	0.157
65	4007	—0.598	—0.261	7.67	0.211
89	4050	—0.602	—0.265	7.98	0.226
b. Series 2.					
5	2690	—0.443	—0.106	7.10	0.331
16	3860	—0.582	—0.245	6.73	0.169
24	4134	—0.611	—0.274	6.61	0.133
29	4174	—0.615	—0.278	6.65	0.131
40	4110	—0.609	—0.272	6.90	0.152
65	4034	—0.601	—0.264	7.67	0.208
89	4072	—0.605	—0.268	7.98	0.223

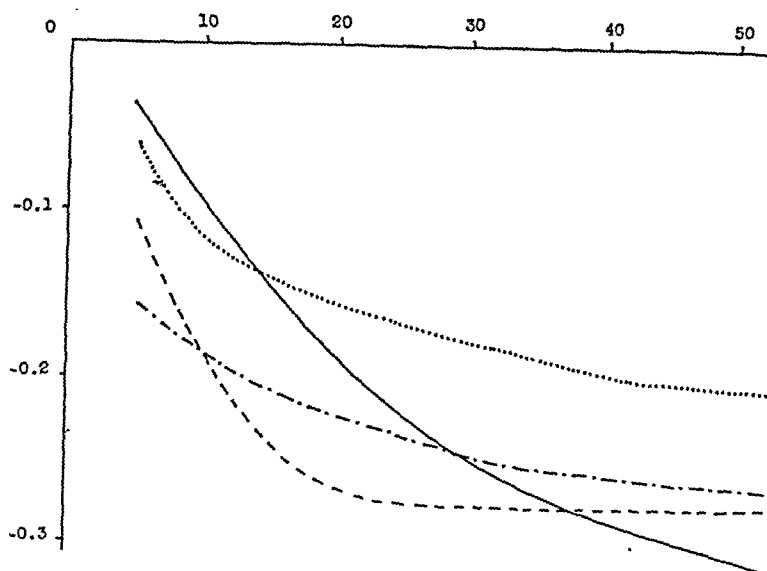


Fig. 10.

Abscissa: t in hours. Ordinate: E_h in volt.

— *Cl. multifementans*.
 - - - *Actinomyces necrophorus*.
 - . - . - *Proteus vulgaris*.
 *Cl. sporogenes*.

The potential curve (Fig. 10) resembles the curve of *Clostridium sporogenes*, but is displaced towards more negative potentials. During the first 24 hours the potentials decrease to a minimum at -0.272 volt, and then increase insignificantly.

Table 26.

Variation of Hydrogen Ion Activity in Culture of *Actinomyces necrophorus*.
 Medium: Potato Broth.

t in hours	pH observed	pH graphically smoothed	$\frac{d \text{ pH}}{dt}$
0	7.50	7.50	-0.125
2	7.14	7.30	-0.073
4	7.10	7.16	-0.053
6	7.07	7.07	-0.041
8	7.05	7.00	-0.035
10	7.02	6.92	-0.033
12	6.97	6.85	-0.031
14	6.89	6.78	-0.029
25	6.57	6.61	0.000
33	6.69	6.71	0.023
38	6.84	6.84	0.028
49	7.14	7.14	0.029
62	7.54	7.54	0.024
73	7.77	7.77	0.016
86	7.87	7.92	0.011
98	8.09	8.07	0.010

Finally another a facultative anaerobe, *Proteus vulgaris*, was cultivated in potato broth. The potentials produced by this bacterium are seen in Table 26.

Table 27.

Oxidation-reduction Potentials in Culture of *Proteus vulgaris* in Potato Broth, cultivated in absence of oxygen.

t in hours	R ₂ in ohm	E _{0.1c} in volt	E _h in volt	pH	E _h + 0.0615 pH in volt
a. Series 1.					
5	3147	—0.489	—0.152	6.75	0.263
16	3564	—0.549	—0.212	6.78	0.105
24	3730	—0.568	—0.231	6.91	0.194
29	3877	—0.584	—0.247	7.00	0.184
40	3890	—0.585	—0.248	7.20	0.195
65	4193	—0.617	—0.280	7.76	0.197
89	4343	—0.633	—0.296	8.25	0.211
b. Series 2.					
5	3203	—0.507	—0.170	6.75	0.245
16	3673	—0.561	—0.224	6.78	0.093
24	3764	—0.571	—0.234	6.91	0.191
29	3913	—0.588	—0.251	7.00	0.180
40	3973	—0.594	—0.257	7.20	0.186
65	4247	—0.623	—0.286	7.76	0.191
89	4370	—0.635	—0.298	8.25	0.209

The potentials produced by this bacterium are more negative than those of *Cl. sporogenes*, but the form of the potential curves is very similar for these two strains.

Table 28.

Variation of Hydrogen Ion Activity in Culture of *Proteus vulgaris* in Potato Broth.

t in hours	pH observed	pH graphically smoothed	$\frac{d \text{ pH}}{dt}$
0	7.50	7.50	—0.285
2	6.98	6.98	—0.180
4	6.76	6.78	—0.029
6	6.75	6.74	—0.011
8	6.72	6.72	0.000
10	6.74	6.73	0.007
12	6.77	6.75	0.011
14	6.76	6.76	0.014
25	7.02	6.93	0.017
33	7.08	7.08	0.020
38	7.11	7.17	0.021
49	7.35	7.40	0.023
62	7.73	7.70	0.023
73	7.97	7.95	0.023
98	8.34	8.34	0.021

Summary.

After a short survey of the electro-chemical conception of oxidation-reduction, a description is given of the apparatus constructed by the author to determine oxidation-reduction potentials in cultures of live bacteria in absence of oxygen. An improvement on the usual technique is obtained by using argon instead of nitrogen as inert gas. The potentials were measured with blank platinum electrodes in bacterium cultures at 37°. The reference electrode was a decinormal calomel cell at 20°. In the tables the potentials E_h referred to the normal hydrogen cell are calculated. The hydrogen ion activities were determined in separate cultures, using a glass electrode and an ionometer.

Oxidation-reduction potentials in cultures of live bacteria are determined during a period of 64—94 hours. The bacteria investigated are partly obligatory anaerobes, partly facultative anaerobes cultivated in absence of oxygen.

The potentials found in cultures of a series of obligatory anaerobes: *Clostridium septicum* (Table 3), *Cl. tetani* (Table 5), *Cl. perfringens* (Table 7), *Cl. sporogenes* (Table 9), *Cl. tetanomorphum* (Table 11) and *Cl. centrosporogenes* (Table 13) cultivated in Difco Bacto Brain Liver Heart Medium show great mutual similarity (Fig. 7). The potentials decrease rapidly during the first 12 hours after the inoculation, then more slowly, and reach a rather constant level after 36 hours.

Two facultative anaerobes: *Staphylococcus aureus* (Table 15) and *Escherichia coli* (Table 17) cultivated under the same conditions (in an atmosphere of argon) showed strikingly different potential curves (Fig. 8). The potentials produced by these bacteria decreased more slowly and uniformly, and no constant level was reached. The potentials remained considerably more negative than those of the *Clostridia*:

	Potential after 60 hours
<i>Cl. sporogenes</i>	—0.32 volt
<i>Cl. tetanomorphum</i>	—0.30 -
<i>Cl. tetani</i>	—0.29 -
<i>Cl. perfringens</i>	—0.28 -
<i>Cl. septicum</i>	—0.28 -
<i>Cl. centrosporogenes</i>	—0.28 -
<i>Escherichia coli</i>	—0.18 -
<i>Staphylococcus aureus</i>	—0.11 -

An attempt was then made to eliminate the sudden fall of the potential in a culture of *Cl. sporogenes* by adding 0.1 per cent of thioglycolic acid to the Brain Liver Heart Medium. The effect was

very conspicuous (Table 19, Fig 9). The potential variation was reduced to a minimum, and the potentials were displaced strongly towards more positive values.

The influence of the medium was further investigated by cultivating *Cl. sporogenes* in potato broth (Table 21). The potential curve found in this case has an intermediate position between the curves obtained with Brain Liver Heart Medium and Thioglycolic Acid Medium, resembling the former more than the latter, but the potato broth has a considerable stabilising effect upon the oxidation-reduction potentials, and the decrease of the potential is slower and more uniform in this medium than in the Brain Liver Heart medium. The potential at the end of the experiment is nearer the potential found in the Brain Liver Heart Medium than that found in the Thioglycolic Acid Medium:

	Potentials after 60 hours
Brain Liver Heart Medium	—0.32 volt
Potato Broth	—0.20 -
Thioglycolic Acid Medium	—0.04 -

Finally a series of bacteria were studied in Potato Broth. *Cl. multifementans* (Table 23, Fig. 10) differs from the other *Clostridia* as the potential shows a continuous decrease during the whole experiment without reaching any constant level. The potentials found in this culture at the end of the experiment are more negative than any of the other potentials observed. *Actinomyces necrophorus* (Syn. Necrosis-Bacillus Bang) (Table 25) gives a potential curve which resembles the curves of the *Clostridia*, as it falls rapidly corresponding to the first 12 hours, and reaches a constant level. The potentials are, however, more negative than those of the *Clostridia* (with the exception of *Cl. multifementans*). *Proteus vulgaris*, cultivated in absence of oxygen, shows a slight and more uniform potential decrease, resembling the curves of the other facultative anaerobes investigated, but the potentials are considerably more negative.

	Potential after 60 hours
<i>Cl. multifementans</i>	—0.32 volt
<i>Proteus vulgaris</i>	—0.28 -
<i>Actinomyces necrophorus</i>	—0.27 -
<i>Cl. sporogenes</i>	—0.20 -

In all of the cases investigated, the oxidation-reduction potential decreases during the growth of the culture, indicating that the cul-

ture becomes more reducing. As the bacterial respiration is connected with oxidations, this result is surprising. During the growth of the bacteria the oxidised products of the respiration will be accumulated, and the concentration of the alimentary substances which are capable of being oxidised, i. e. are present in reduced form, will be diminished. The oxidation-reduction potential is determined by the formula

$$E = E_0 - 0.0001985 \frac{T}{n} \log \frac{[\text{Oxidant}]}{[\text{Reductant}]}$$

(see Equation 10 and the corresponding footnote), and accumulation of the oxidant and decrease of the reductant consequently must cause an increase of the potential instead of the observed decrease. The effect may be explained in the following way: As the respiration of the bacteria takes place under anaerobic conditions, the oxidations cannot be realised by means of oxygen. The oxidative reactions of the respiration therefore are realised by means of hydrogen acceptors which are reduced at the sacrifice of the alimentary substances which are oxidised. The hydrogen acceptors accordingly are accumulated in reduced form. The oxidation of the alimentary substances is irreversible, and the oxidation products do not influence the potential essentially. The oxidation-reduction potential will be mainly determined by the reversible equilibrium: Reduced Hydrogen Acceptor \rightleftharpoons Oxidised Hydrogen Acceptor, and as the hydrogen acceptor is reduced by the respiration, the potential will decrease.

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ON THE OCCURRENCE IN BULL SPERM OF CERTAIN »MEDUSA FORMATIONS« DERIVED FROM THE EPITHELIUM OF THE EFFERENT DUCTS OF THE TESTIS

By *Erik Blom*.

(Received for publication April 20th, 1944).

On systematic morphological examination of specimens of sperm from over 200 normal breeding bulls carried out for the purpose of determining the amount of abnormal sperm cells in normal bull sperm, I have made some observations which presumably may be of general interest although they have no direct bearing on the main purpose of the examinations.

The smears employed for these examinations were made from the sperm specimens immediately after the collection of the sperm (artificial vagina). Later the smears were stained in the laboratory after a method given by me (2) with a 1% methyl violet solution with pH of about 10, and mounted in neutral Canada balsam. The differential count of the sperm cells was carried out at a magnification of about 1800 \times , the morphological features being estimated on a total of 500 sperm cells distributed over several areas in each specimen.

After I had worked for some length of time with such differential counts, I met with a very peculiar »form of sperm cells« which I had not seen described anywhere, and it could not be classified after any of the groupings usually employed for the abnormal forms of sperm cells. The form here concerned consisted in a rather deeply staining base, a little smaller than the head of a normal sperm cell, and a bundle of filiform structures — about 20 threads — arose from this base. All these threads were very thin and measured only about 10 μ in length, that is, they were much shorter than the tail of a normal bull spermatozoon, which measures 60–70 μ .

In a preceding paper of 1943 (3) on the morphology of the bovine spermatozoa, as a curiosity a picture of this »Medusa formation« was included — the structure was given this designation because



Fig. 1.*)



Fig. 2.

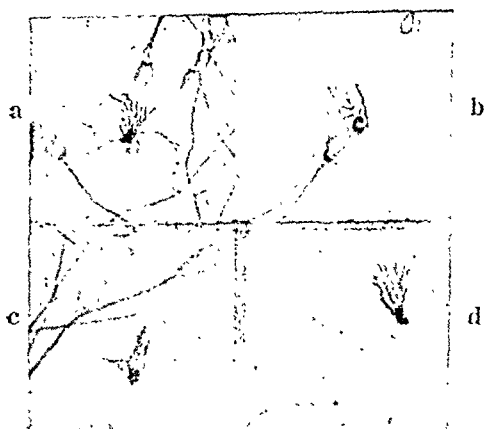


Fig. 3.

of its characteristic appearance — under the probability diagnosis: conglomerate of underdeveloped tails? or possibly a detached spermatoblast?

Now I had become interested in the occurrence of polycaudate sperm cells in general and in this apparently new spermatozoal form in particular. In 200 differential counts carried out in the following period with the usual technique, 9 additional specimens presented a morphology reminding of the above-mentioned to such an extent that it would only be reasonable to reckon all 10 as belonging to the same group.

The 10 Medusa formations were observed in specimens of normal ejaculates from 10 healthy breeding animals. These formations all consisted of a deeply staining base with a diameter of 2—5 μ ; in several cases this base was subdivided distinctly into a number of dark

Fig. 1. Four different Medusa formations. In *a* the basal part is divided into granules which are arranged in a semicircle; in *b*, *c* and *d* the deeply stained base is practically triangular.

Fig. 2. Four different Medusa formations. In *a* and *d*, very distinct granules at the base; in *b* the granules are arranged in a ring.

Fig. 3. Four different Medusa formations. In *b* distinct granules at the base.

*) All the microphotos (magnif. $\times 600$) are taken with a Leica apparatus.

I am greatly indebted to Dr. Anker Hansen, V. S. for his valuable assistance in the photography.

roundish granules arranged in a semicircular — or, in one instance, circular — pattern. From this base, groups of thin threads, »tails«, were projecting in numbers varying from 8—10 threads to over 30. In the cases where the dark granules were placed in a single cluster or in a semicircle, all the tails were pointing in the same direction, whereas in the specimen where the granules were ranged in a circle, the tails were grouped in bundles radiating in various directions. As a characteristic feature, the variations in the length of the »tails« kept within the range of 10—15 μ (cf. Figs. 1—3).

The most probable explanation of the appearance of these formations seemed to be that here we were dealing with an abnormal form of sperm cells with multiple division of the tail anlage, or, in other words, of the centrioles from which the axial filament of the sperma-

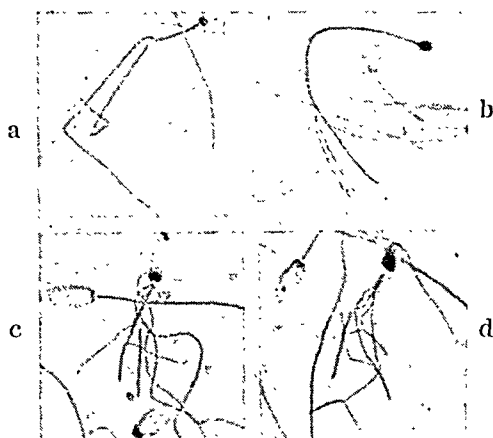


Fig. 4.



Fig. 5.

Fig. 4. *Abnormal forms of sperm cells for comparison.* a, pinhead form with two tails. b, pinhead form with three tails. c, 4-tailed sperm cell. d, 5-tailed sperm cell.

Fig. 5. *Various findings in smears of teased efferent ducts.* a, solitary »roundish« Medusa formation (cf. Fig. 2, d). b, »Medusa« with triangular base (cf. Figs. 1, b, and d). c and d, isolated epithelial cells with the cilia attached.

Fig. 6. *Epithelium of the efferent tubules of the testis in bull.* Thickness of section: 5 μ . Azan stain. (Specimen prepared by Anker Hansen). About every other cell shows a distinct equipment with cilia. One cell is filled with secretory granules.

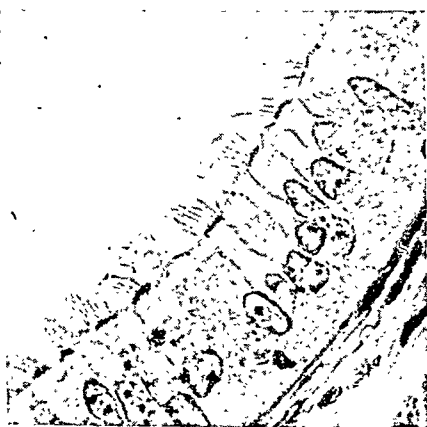


Fig. 6.

tozoal tail is assumed to arise. Thus the round, dark, granules in circular arrangement would result from multiple division of the hyperplastic cell center. This hypothetical explanation, I thought, found support in the observation that several of these specimens also showed deformed spermatozoa with diminutive heads that apparently consisted mostly of one or more markedly enlarged centrioles provided with one or two well-developed tails («pinhead form») (Fig. 4 a).

Another interesting observation was that several specimens of sperm presented some markedly deformed or incompletely developed spermatozoal heads with 3, 4 and — in a few cases — 5 tails (Figs. 4 b, c, d). But even though the tails of these sperm cells often were somewhat entangled, they always presented the length characteristic of bovine sperm cells: 60—70 μ , with division into middle piece and principal part.

So, in the question as to whether the Medusa formations were to be looked upon as polycaudate sperm cells, it had not been practicable in a convincing way to establish a gradual developmental series from ordinary sperm cells with two tails, over sperm cells with 3, 4 or 5 tails, to the relatively rare Medusa formation. In the series there was a wide gap between a tail length of 60 μ and that of 10—15 μ , as no intermediate sizes were seen.

It was a mere matter of chance that I found the explanation of these Medusa formations. For an entirely different purpose I was investigating some smears from various parts of epididymis, which had been teased and stained with methyl violet, when in a specimen I met with a great number of the peculiar Medusa formations (Fig. 5). This particular specimen came from the efferent ducts of the testis. Here the Medusa formations were lying free, in rows of 3—4 or singly, in the neighbourhood of groups of typical epithelial nuclei surrounded by more or less torn cytoplasm: the Medusa formations could simply be nothing but isolated fragments of the ciliated coat with which the epithelial cells of these ducts are provided. Comparison of these specimens with stained sections from the corresponding part of the epididymis confirms this observation (Fig. 6).

Thus the Medusa formations were identified as isolated fragments of the ciliated coat of the efferent ducts of the testis, together with a little cytoplasm from the epithelial cells. No doubt they have been carried through the efferent ducts and the ductus epididymidis by the stream of sperm cells passing through these ducts, and then ejaculated later together with the sperm cells. The dark round granules were simply the basal bodies of the cilia, and the constant shortness of the »tails« was now quite natural, the »tails« being cilia. When the Medusa formations in several cases were more roundish than fragments of the ciliated coat *in situ* or even rolled, it may perhaps be explained as a reaction to the surroundings, so that the ciliated

surface previously facing the lumen of the duct now became curved (Figs. 2 b, 2 d, 5 a).

How is the finding of these rather rare formations to be interpreted?

As it seemed reasonable to assume that the presence in the ejaculate is a physiological phenomenon, I tried to see whether this would hold true by thorough examination of 3 specimens from 3 healthy bulls whose sperm had not shown any Medusa formation previously. These specimens were examined very thoroughly with the one aim of finding such forms, about an hour being spent on the examination of each specimen. The result was that one specimen presented two Medusa forms, another presented 1 Medusa form, while the third showed no such structure. No doubt the demonstration of such Medusa forms is merely a question of perseverance and care.

With one exception all the bulls whose sperm showed Medusa formations have had a relatively short period of abstinence varying from 1 to 3 days. Only one Medusa formation was found in the ejaculate from a bull which was stated to have had an abstinence period of 2 weeks. This medusa formation is not included in the illustrations, as it was not well preserved.

As the limited number of observations in the present material naturally makes it somewhat uncertain on this basis to estimate the probable occurrence of Medusa formations in bull sperm in general, the following calculation is to be taken merely as an orientation:

As mentioned, 10 Medusa formations were found in 200 differential counts of 500 sperm cells — *i. e.*, a total of 100,000 sperm cells — and this means that one may have to go through about 10,000 sperm cells in order to find one »Medusa«.

Accordingly, it may appear as if here we are dealing with a rare formation which really is of interest only as a curiosity. But perhaps this view may have to be modified somewhat if we try to estimate how many of these detached fragments of the ciliated coat are evacuated together with a single normal ejaculate.

Reckoning the average sperm concentration in the bull to be about 1 million per cmm., and the volume of the ejaculate = 5 cc., and supposing the occurrence of one Medusa formation per 10,000 sperm cells holds true, we shall have about 500,000 Medusa formations per ejaculate. So, evidently, the production of these particular formations must be quite considerable — even though their occurrence and genesis hitherto have been unnoticed.

As to the search for these formations, it will be appropriate here to mention that the methyl violet stain here employed means a very intense overstaining of all other cells and cell elements than sperm cells — that is, of the Medusa formations too. So it is rather doubtful whether these fragments of the ciliated coat, which really takes the

stain but poorly, would have been noticed at all on employment of one of the usual histological staining methods.

Even if the Medusa formations under practical conditions should be counted as abnormal sperm cells, this would mean a quite insignificant source of error, in view of their normally rare appearance. The possibility is not excluded, however, that under certain circumstances an ejaculate may present a relatively large amount of these detached fragments of the ciliated coat, and hence it may be of some importance to know the particular origin.*)

The knowledge of the Medusa formations here described and the demonstration of their origin from the epithelium of the efferent ducts of the testis calls to mind the discussion carried on among histologists ever since the 90's about the structure and function of the very peculiar ciliated epithelium of these ducts. This discussion has not come to an end yet as directly contradictory descriptions are still being published in the various recent histological textbooks.

Several investigators — above all, *Schaffer* (6, 7) (1892) — have described in man a single layer of epithelial cells lining the efferent tubules of the testis as consisting of two distinctly different types of cells: 1) non-ciliated cells with light cytoplasm, and 2) ciliated cells with dark cytoplasm. Of these two types, the non-ciliated cells are assumed to be secreting cells proper, whereas the main task of the ciliated cells should be the further transport of the secretion and sperm cells. *Aigner* (1) (1900) investigated the structure of the epididymis in rabbit, rat, cat and horse and found the efferent tubules of the testis in these species to be lined with an epithelium which in all essentials corresponds to the findings reported by *Schaffer* in man. Like *Schaffer*, also *Aigner* asserted emphatically that here we are dealing with two different forms of cells.

In 1897 *Hammar* (4) described the corresponding epithelium in dog. After a thorough analysis of the highly varying secretory features encountered in various lumina within the same section, he arrived at the conclusion that all the cells lining these tubules belong to one and the same form, the appearance of which is merely subject to great functional variations. *Hammar* reckoned the secretion as being of the merocrine type and divide the secretory cycle into the following phases:

1. *Resting phase*: All the cells are provided with kinocilia. The epithelium reminds most of a quite indifferent ciliated epithelium.
2. *Storing phase*: Some of the cells in the tubules (often about every other cell) become filled with secretory granules.

*) *Rich. Hammen* M.D., who through several years has carried out numerous differential counts on human sperm, has told me personally that he has observed similar formations in a few specimens of human sperm.

3. *Discharging phase*: In the beginning of this phase the storing cells lose their cilia and discharge their secretory granules, whereafter they present a light, translucent and fine-mashed appearance.
4. *Recreation phase*: The »emptied« cells contract, and probably a new growth of cilia commences, so that the cell again enters the resting phase as a very low ciliated cell.

Without claiming that the findings here reported have to be taken as conclusive evidence in support of *Hammar's* theory, I still think that the assumption of such an alternation between the ciliated stage and the secretory stage in the epithelial cells of the efferent tubules of the testis is the most reasonable explanation of the occurrence of the Medusa formations here described.

Addenda in the Proof.

Lately I have had the opportunity to examine some specimens of sperm from 5 breeding stallions, and I have been able to demonstrate several Medusa formations in each of these specimens. Generally these formations in the sperm of the stallion are larger and apparently more frequent than in the bull.

Particularly many Medusa formations were found in the sperm of a stallion examined after this animal had been employed for breeding purposes at a high rate for some length of time. As an artificial vagina for the stallion could not be obtained at its quarter, the following two specimens were sent to the laboratory: I) a specimen of sperm obtained directly from the penis of the stallion as it left the mare after completed covering; II) a specimen taken from the vagina of the mare immediately after this act of covering. In specimen II Medusa formations could be demonstrated without difficulty, though not in any great number. In specimen I, on the other hand, representing the very last part of the ejaculate, without any admixture of vaginal secretion, on an average one Medusa formation was seen in each microscopic field at a magnification of 300, and after counting several fields this frequency was estimated to correspond to about 1 Medusa formation per 500 sperm cells.

Summary.

On morphological examination of specimens of sperm from 200 healthy Danish breeding bulls, a so-called »Medusa formation« was observed in 10 cases.

These hitherto unnoticed bodies are about as large as, or a little larger than, the head of a normal sperm cell, consisting of a deeply staining base with 10—30 filiform projections.

These structures are found to be detached fragments of the ciliated

coat covering the epithelial cells which line the efferent tubules of the testis.

On the basis of the present material the general occurrence of these structures in the bull is estimated roughly to be about 500,000 per ejaculate.

The demonstration of these bodies in the sperm is taken to lend support to the theory advanced by *Hammar* in 1897 concerning the phases of the secretion in the efferent tubules of the testis.

Quite corresponding formations have been demonstrated later in specimens of sperm from stallions.

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ON THE DISTINCTION BETWEEN PATHOGENIC AND NON-PATHOGENIC STAPHYLOCOCCI

By *Johs. Boe*, M. D.

(Received for publication May 10th 1944).

As a rule the staphylococcus strains isolated from pathological processes exhibit characteristics which clearly reveal the pathogenic nature of the microbes, and the etiological relation to the disease is clear.

But the frequent occurrence of staphylococci on normal skin and mucous membrane, and their abundance in the air does that these microbes may also be found in pathological processes where they have not necessarily played an etiological role. And this uncertainty is accentuated by the fact that we are not certain of the pathogenic characteristics of staphylococci and that the border line between pathogenic and non-pathogenic staphylococci is not sharp.

There are many methods for determining the pathogenicity of a staphylococcus strain: examinations of pigment formation, hemolysin production (toxin), fermentation of sugars (mannite), liquefaction of gelatin, plasma coagulation, the crystal violet reaction, to name only the most important. In addition there are the direct animal experiments and also the serological reactions.

Opinion is divided as to these methods of investigation. Individually none of the biochemical reactions is entirely reliable. Animal experiments for the determination of the pathogenicity of staphylococci are also rather uncertain. And as for serological diagnosis within the Genus *Staphylococcus*, there is still a long way to go before it attains the same accuracy as for some other bacteria.

The present publication is an attempt to evaluate the biochemical reactions in determining the pathogenicity of staphylococci. The distinction between pathogenic and non-pathogenic staphylococci is further attempted by help of serological reactions.

Since *Rosenbach* made the distinction between yellow and white staphylococci, and since hemolysis was found to be a property most frequently related to the pathogenic staphylococci, the term »yellow, hemolytic staphylococci« has been practically synonymous with pathogenic staphylococci. As

a matter of fact both the production of pigment and hemolysin are unreliable characteristics (*Julianelle*, 1922) and most investigators are now agreed (cf. *Chapman* and co-workers, 1934 and *Flaum*, 1938). However there are some who regard the production of hemolysin as a basic property which should form the foundation of a classification (*Cowan*, 1938).

The capacity to coagulate citrate plasma seems to be a characteristic property of the pathogenic staphylococci. And this reaction is now regarded as the best of the biochemical »pathogenicity reactions« (v. *Daranyi*, 1926 and 1935, *Gross*, 1928, *A. Hallmann*, 1937, *Fairbrother*, 1940, *Kourilsky and Mercier*, 1942) but it may also fail.

Of the capacity of staphylococci to ferment sugars, only the reaction to mannite is considered in this connection. *Julianelle* (1937) who tried to find a method which could take the place of the rather cumbersome serological distinction which he had found between pathogenic and non-pathogenic staphylococci, discovered that the fermentation of mannite failed in only 5 %. This has been confirmed by subsequent investigations (*Thompson and Korazo* 1937, *Chapman* and co-workers 1937, *Flaum* 1938, *Kourilsky and Mercier* 1942). However *Flaum* emphasizes that it is important that the reaction is read after an interval of not more than 2 days.

Chapman and Berens (1935) have attempted to distinguish between pathogenic and non-pathogenic staphylococci by means of selective dye media. They recommend inoculation in crystal violet agar where the pathogenic staphylococcus grows with a violet or yellow color. *Kourilsky and Mercier* (1942) have also found that this is a valuable reaction. Later *Chapman* and co-workers (1937) recommended a brom thymol blue medium, which, at the same time as it simplifies the primary isolation, also gave a determination of the pathogenicity of the staphylococcus, as only pathogenic staphylococci could grow in this medium. However there seems to be no agreement as to the value of these last two reactions (*Schmidt* 1940).

Rabbits have usually been used in animal experiments to determine the pathogenicity of staphylococci. These animals have considerable resistance to staphylococcus infections, and it varies a good deal from one animal to another. Virulent strains cause a fatal pyemia, but strains isolated from pathogenic processes and which certainly would cause disease in humans may be avirulent for animals. Intravenous injection of staphylococci in rabbits for the determination of pathogenicity is therefore of limited value (*Chapman* and co-workers, 1937, *Flaum* 1938).

Attempts have also been made to solve the problem of pathogenicity by subcutaneous injection (v. *Daranyi* 1926) and by intracutaneous injection in rabbits (*Gross* 1928). It is true that most pathogenic strains cause more extensive lesions than the non-pathogenic, but here there is also the wide variation in the resistance of the individual animals. And the extent of the reaction is also to a large degree dependent on the capacity of the strain to produce toxin (on intracutaneous injection dermonecrotic toxin). Toxin production is found only among the pathogenic strains, but only a few of these are good toxin producers.

The serological methods have naturally been used to a constantly increasing extent to distinguish between the pathogenic and non-pathogenic staphylococci and to make a more accurate classification within these two groups. The first convincing results were obtained by *Julianelle* (1922) when he was able to classify 25 strains into 3 groups by means of agglutination and agglutinin absorption. But he found no relation between the serological classification and the other properties of the strains. *Seedorff* (1924) found 13 types among his strains by complement fixation. However the majority of the strains belonged to two types of which one included strains from pathogenic processes and the other white strains from otitis media and acne.

Danbolt (1931), who used agglutination, found that the pathogenic strains which he examined, mostly from furuncles, were different from the white staphylococci he found in acne. Within the pathogenic group there were small differences.

Julianelle and Wiegard (1935) seem to be the first to demonstrate a distinct serological difference between the pathogenic and non-pathogenic strains. They showed that there are type specific polysaccharides in the staphylococcus, and by precipitation they were able to classify the strains into a type A which included the pathogenic strains and a type B which included the saprophytic strains. These results have been largely confirmed by subsequent investigators (*Thompson and Khorazo* 1937, *Hegemann* 1937, *Cowan* 1938) but they have found additional types.

A serious obstacle in the work with serology of staphylococci has been the difficulty in obtaining a stabile antigen for agglutination. *Yonemura* (1936) claims to have obtained a stabile antigen by treating the microbes with antiformin ad modum *Kuroda* (*Aoki* 1937), and by absorption he was able to classify his strains into 9 types.

Among more recent investigation it seems that *Krag Andersen* (1943) has advanced an important step further in the investigation of the serology of staphylococci. By means of trypsin treatment she obtained a stabile, reliable antigen. And by absorbtion experiments she was able to demonstrate that staphylococci isolated from pemphigus neonatorum were different from other pathogenic staphylococci. And she was able to find this specific pemphigus staphylococcus again in the nose and throat of carriers. This seems to have opened the way for a classification of staphylococcus antigens.

As for the limitations of the Genus *Staphylococcus*, *Bergey* (1939) includes only those species which liquefy gelatine. In accordance with this system, only those strains which liquefy gelatine are included in the present investigation, as the systematic position of the gelatine-negative strains must be regarded as uncertain.

Personal material.

The material here presented consists of 509 strains of staphylococci, isolated from various staphylococcus diseases in humans, as well as strains from normal skin and mucous membrane, and some strains isolated from air.

All the strains were tested for pigment and hemolysin production, plasma coagulation and mannite fermentation. We also studied the growth of some of the strains on crystal violet agar and brom thymol blue agar, but the value of these media seems to be so little that we omitted them as the investigation progressed. Some strains were tested in animal experiments by intravenous, intracutaneous and subcutaneous injections. 223 strains were serologically tested.

Technique.

The strains were isolated on rabbit blood or human bloodagar plates. All the strains had the typical microscopic appearance and all were Gram positive. Inoculation from the first pure culture was used

for the biochemical reactions, so that dissociation phenomena could be avoided.

Pigment production was studied on agar and bloodagar after 24 hours in a thermostat at 37° C and thereafter at room temperature for 6 days. The amount of pigment formed was not graded but was recorded as + or —. In rare cases it may be difficult to determine whether a strain forms pigment or not.

Hemolysin production. Hemolysin production on solid media is determined by inoculation on 5 % rabbit or human bloodagar plates. Incubation at 37° C for 24 hours, and thereafter 24 hours at room temperature. Hemolysin formation in solution was determined in the centrifugate or Zeitz filtrate from a veal broth (pH 7.0) which was incubated for 8 days after inoculation. The solution in progressive dilutions and in a fluid quantity of 1 cc was added to 1 cc 1 % washed rabbit blood corpuscles. Result recorded after 1 hour in a water bath at 37° C and thereafter 20 hours in a refrigerator at + 4° C.

Plasma coagulation. 20 cc rabbit blood was added to 20 cc 2.5 % sodium citrate. The plasma was distributed in tubes after centrifuging, 0.5 cc in each. A loopful of 24 hour old agar culture was then added. The results were read after 1—4 hours in a water bath at 37° C.

Mannite fermentation was determined in the following medium. 4 gr mannite and Andrades indicator were added to 400 cc peptone water (1 % peptone Witte, 0.5 % NaCl). This was sterilized by live steam for 20 minutes after having been filled in tubes. After inoculation from agar cultures and incubation, the results were read after 48 hours.

Crystal violet agar and *brome thymol blue agar* which were used during the early part of the investigation, were prepared according to the method given by *Chapman and Berens* (1935) and *Chapman* and co-workers (1937).

Preparation of immune sera. Rabbits were immunized by prolonged and strong immunization intravenously with formalin-killed staphylococci. The rabbits were tapped 7 days after the last injection. Sera for precipitation were stored sterile in a refrigerator with no addition of any kind. Equal parts of glycerin were added to the sera for agglutination.

Precipitation. The antigen was prepared by two methods.

1. According to *Hegemann's* method (*Hegemann* 1937): Three 24 hour old agar slant cultures are suspended in 5 cc physiological saline solution and boiled for 10 minutes. 1.5 cc n/5 HCl is then added and this is again boiled for 15 minutes. Then neutralized with n/4 NaOH with methyl orange as indicator. Thereafter centrifuging until the fluid is perfectly clear. The reaction is set up in capillary tubes where the extract is taken in first and then the serum. There is usually precipitation after 5 minutes. The result is recorded after

1 hour in a thermostat at 37° C. and thereafter 24 hours in a refrigerator.

2. Three 24 hour old agar slant cultures were suspended in 5 cc saline. The suspension was then placed in a shaking machine for a half hour and then centrifuged until clear. The reaction was set up as above.

Agglutination. Agglutinin titration was carried out with live, heat-killed and formalin-killed antigen, as well as antiformin treated antigen prepared as follows: 24 hour old agar cultures are suspended in saline to which is added equal parts 2 % antiformin. The suspension stands for a half hour in a thermostat at 40° C. After centrifuging the antigen is washed three times with saline and is then diluted with saline to the desired dilution. The reactions were usually recorded after 2 hours in a water bath at 37° C. and thereafter 24 hours at room temperature, but recording after 24 hours in a water bath at 37° C. was also tried.

The origin of the strains is presented in Table 1. Here the strains

Table 1.
The origin of the strains and their biochemical reactions.

		No of strains	Pigment		Hemolysis		Plasma-coagulation		Mannite fermentation	
			+	-	+	-	+	-	+	-
I.	Furuncles, Carbuncles.	50	43	7	39	11	39	11	43	7
	Pyodermia	8	8	0	5	3	7	1	7	1
	Impetigo	4	3	1	2	2	3	1	3	1
	Sykosis.....	8	8	0	7	1	8	0	8	0
II.	Rhinitis, sinusitis	79	59	20	65	14	54	25	57	22
	Angina	5	4	1	5	0	5	0	5	0
	Lung affections (bronchitis, pneumonia, epyema abscess, gangrene	52	48	4	41	11	45	7	46	6
	Otitis, mastoiditis.....	8	2	6	5	3	1	7	2	6
III.	Conjunctivitis.....	14	10	4	9	5	7	7	8	6
	Sepsis. pyemia	14	12	2	11	3	13	1	13	1
	Osteomyelitis	11	9	2	8	3	8	3	7	4
	Abscesses, sores	64	60	4	52	12	58	6	59	5
IV.	Pyartros.....	6	6	0	6	0	6	0	6	0
	Urinary infections	40	23	17	23	17	21	19	28	12
	Feces.....	2	2	0	1	1	1	1	2	0
	Normal skin.....	36	20	16	14	22	3	33	14	22
V.	Normal throat and nose	83	53	30	57	26	50	33	50	33
	Air	25	5	20	11	14	3	22	6	19
		509	375	134	361	148	332	177	364	145

are classified according to the pathological processes and the localizations from which they are isolated, in order to see whether there is any relation between the properties of the strains and their origin.

No such relationship can be found. It also appears from the table that strains could be isolated from almost all kinds of definite staphylococcus infections which according to their biochemical properties were non-pathogenic. It appears from the table that plasma coagulation was the property which was most seldom positive so that, when it was positive, it should be regarded as the most reliable characteristic of pathogenicity.

Table 2 shows the mutual relationship of the biochemical reactions.

Table 2.
The mutual relation of the biochemical reactions.

	Pigment		Hemolysis		Plasma coagulation		Mannite fermentation	
	+	÷	+	÷	+	÷	+	÷
No of Strains	375	134	361	148	332	177	364	145
Pigment								
+			311	64	326	49	338	37
÷			50	84	6	128	26	108
Hemolysis								
+					290	71	303	58
÷					42	106	61	87
Plasma coagulation								
+							324	8
÷							40	137

It is seen that the biochemical reactions do not always follow each other, but here also, plasma coagulation seems to be the most reliable reaction, as the coagulase positive strains in 98.4 % and 97.2 % were also pigment producers and mannite positive respectively. Hemolysin production was more uncertain than the other reactions in our investigations as we found a number of apathogenic (white, coagulase and mannite negative) strains which gave hemolysis on plates, while some definitely pathogenic strains failed to hemolyse. Hemolysin production in solution was also a poor reaction in this respect. It is true that hemolysin was found in solution only in the pathogenic

strains, but many definitely pathogenic strains produced no soluble hemolysin.

Of the 509 strains studied, there was complete agreement as to the biochemical reactions in 345 strains, i. e. in 67.6 %, cf. Table 3.

Table 3.

The results of the four »pathogenicity reactions« employed in strains from pathogenic processes and strains from the normal organism and air.

	Strains from pa- thogenic processes	Strains from nor- mal and mucous membrane and from air	Total
All 4 reactions positive	225	51	276
3 » » »	52	7	59
2 » » »	20	14	34
1 reaction positive	47	34	71
All 4 reactions negative	31	38	69
	365	144	509

Where there were deviations, these were in most cases due to pigment formation or hemolysin production. Of the 59 cases where 3 of the 4 biochemical reactions were positive, and it is thus expected that the strain in question was pathogenic, the 4th reaction which was negative was hemolysin production in 50 cases, plasma coagulation in 5 cases and mannite fermentation in 4 cases. Pigment formation was positive in all of these, but was negative in the other groups and was positive where the other reactions were negative.

Of the 134 unpigmented strains in this material, 129 could be classified according to *Bergey* (1939) as 106 of the strains were classified as *Staphylococcus epidermidis* (fermented saccharose, but not mannite and raffinose). 21 strains were *Staphylococcus albus* (fermented saccharose and mannite but not raffinose) and 2 strains were *Staph. pharyngis* (fermented saccharose, mannite and raffinose). In this material *Staph. epidermidis* occurred more frequently in the throat than *Staph. pharyngis*, so the name has little relation to the localization of the microbe.

Serological investigations.

We prepared a total of 13 immune sera by immunization with strains of various origin and some differing properties. Sera were prepared from strains from furunculosis (2), carbuncle (1), osteomyelitis (1), pyarthrosis (1), empyema (1), sepsis (2), abscess (1), rhinitis (1), normal throat (1) and normal skin (1).

By means of these sera we classified our strains in 2 serological groups, as sera from pathogenic staphylococci precipitated extracts

Table 4.

The relation between the serological group and the biochemical reactions in 223 staphylococcal strains.

	Group A.	Group B.
All 4 reactions positive	134	3
3 " " "	29	0
2 " " "	8	5
1 reaction positive	6	15
All 4 reactions negative	0	23
	177	46

of pathogenic strains, and sera prepared with apathogenic strains precipitated extracts of apathogenic strains. There were few exceptions from this rule (cf. Table 4) but there was not complete agreement between the result of the serological examination and the biochemical reactions. The 3 strains which showed all 4 reactions positive but which were serologically classified in group B originated from an angina (298), from air (468) and from pyoderma (494). We prepared an immune serum with one of these strains (298) which reacted like the other sera prepared with pathogenic strains with respect to precipitation. In the agglutination experiments (see below) this serum also reacted like the others. The explanation of this failure of the precipitation reaction may be that with the method here used for the preparation of the extract, the precipitogen may occasionally be destroyed. The results of the precipitation reactions were clear, the reactions were easily read off.

The water obtained after shaking the staphylococcus suspension in the shaking machine also gave pronounced precipitation in the majority of cases, and this was a simple method of determining to which serological group a staphylococcus strain belonged. However it was not as accurate as when the other extract was used.

By extensive agglutination and agglutinin absorption tests we attempted a more accurate serological classification. We shall only give a brief account of our experiments as they did not lead to uniform results. The greatest difficulty was, as mentioned above, to obtain a stabile antigen. With the methods we employed the results were not so constant from one time to another that we found any basis for classification. We found that a serum gave a high titre with the homologous strain; with most of the other strains with which the antigen reacted, the titre was lower. In a few cases, by means of absorption experiments, we were able to determine that some strains were serologically identical, but in most cases we found all the transitions in the antigen structure, and the limitations were not so definite that we could set up serological types on this basis.

Animal experiments.

A number of our strains were tested by experiments on rabbits with intravenous, subcutaneous and intracutaneous injections, and we also tried percutaneous application. The last method gave no results. The other methods showed that there was considerable difference between the individual strains with regard to the development of pathological processes. On subcutaneous, and even more on intracutaneous injection we found that the reactions produced were even more dependent on the reactive capacity of the animal (allergy) than on the virulence of the individual strains. These investigations on the significance of allergy in staphylococcus infections will be published separately.

Summary.

There seems to be no sharp limitation between pathogenic and apathogenic staphylococci. And as these organisms are often found on the healthy organism and in the air, it is often difficult to be certain as to whether an isolated staphylococcus has been of pathogenic significance in the pathological process from which it is isolated.

Opinion is divided as to the value of the biochemical reactions in general use for determining the pathogenicity of staphylococci. Individually none of them is reliable.

The object of the present investigation was to evaluate these biochemical reactions in the determination of the pathogenicity of staphylococci. The results were then compared with serological tests, with animal experiments and with the pathological processes and the localizations from which the strains were isolated.

509 strains were isolated from various staphylococcus infections in humans, from normal skin and mucous membrane and from air.

No staphylococci with particular characteristics for any pathological processes or localizations could be found.

Plasma coagulation was found to be the most reliable sign of pathogenicity, and next after this mannite fermentation. Pigment formation and hemolysin production were very unreliable properties.

The crystal violet reaction was also unreliable, and cultivation on brome thymol blue agar did not seem to afford any advantages, either with respect to the primary isolation or the determination of pathogenicity.

Animal experiments afford no reliable basis for distinguishing between pathogenic and apathogenic staphylococci, as many definitely pathogenic strains are only slightly virulent for animals. On subcutaneous and intracutaneous injection, the reactive capacity of the animal seems to be just as significant as the pathogenicity of the strains.

By precipitation experiments the strains could be classified into a group A which included the pathogenic strains and a group B which included the apathogenic strains. In a few cases there was disagreement between the biochemical reactions and the serological properties of the strains.

Agglutination and agglutinin-absorption showed that there were all transitional forms between the antigen structure of the strains, but the difficulties involved in obtaining a stabile antigen make the results of these experiments unreliable. Antiformin treatment of the antigen seems to be no better than heat-killed or formalin-killed antigen.

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AUTOPSYIC EVALUATION OF THE CLINICAL DIAGNOSIS »CANCER OF THE STOMACH«, WITH SPECIAL REFERENCE TO ITS VALUE TO THE CANCER STATISTICS.*)

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(Received for publication May 19th, 1944).

Introduction.

To all clinicians and pathologists it is a well-known fact that the clinical diagnosis »cancer of the stomach« does not always correspond to the autopsy findings. How often the clinical diagnosis disagrees with the autopsy diagnosis, however, has been investigated but incompletely. This question has become of particular interest in Denmark after the institution of the Danish Cancer Register, to which every instance of malignant tumors in this country is notified. One of the forms of cancer about which it is most difficult to obtain exact statistical information is cancer of the stomach. A positive histological verification of this diagnosis in vivo is obtained only in the not particularly frequent cases in which tissue is removed operatively for examination, or in which the presence of tumor cells can be demonstrated on microscopic examination of the stomach contents. And even in Copenhagen where the frequency of post-mortem examination is higher than in most other places throughout the world, autopsy is performed only in about one-half of the cases, as about 60 % of the patients with this lesion die in the hospitals and the rate of autopsies here is about 80 %¹. So, to a large extent, the Cancer Register has only the clinical diagnosis to go by. The aim of the work here presented, therefore, has been to elucidate the reliability of the clinical diagnosis — *i. e.*, every diagnosis in vivo, that is, to see how often the clinical diagnosis »cancer of the stomach« is confirmed or refuted by the autopsy findings.

*) The studies here reported were carried out with the aid of a grant from the Danish National Association for the Combating of Cancer.

Material.

The present material comprises 9,702 autopsies performed on adults (≥ 15 years) who died in the Surgical and Medical Departments of Kommunehospitalet, Copenhagen, within the period of 1915—1935, inclusive. I have gone through each of the nearly ten thousand autopsy records in order to make sure that this investigation actually covered all the cases of cancer of the stomach verified by autopsy as well as assumed clinically. In 1069 of the cases information is missing about the clinical diagnosis made by the hospital physicians prior to the autopsy. These cases (11.0 %) are therefore omitted from these studies. In most of the cases where the clinical diagnosis was missing the patient had died within 24 hours after his admission to the hospital.

In the remaining 8,633 cases, on the other hand, the diagnosis made prior to the autopsy is put down in the autopsy records. By this I mean only the diagnosis made by the clinicians in the hospital.

That these clinical diagnoses as a rule are available prior to the autopsy, and are recorded together with the autopsy findings must be said to be a very valuable tradition that ought to be kept up where it has been observed and introduced where it is not yet adopted. In the working up to the materials it affords a most valuable control of the value of the clinical diagnoses.

Analytical Method.

The investigation is introduced with a count of the number of patients who died in the period here concerned under the clinical diagnosis cancer of the stomach. From this number we have to subtract the patients who on autopsy were found not to be suffering from cancer of the stomach but from some other disease (the clinical overestimation). The result of this gives the number of patients with the correct clinical diagnosis cancer of the stomach. To this number we have to add the cases in which cancer of the stomach was revealed by the autopsy but had not been recognized clinically (clinical underestimation). The final results is the total number of cases of cancer of the stomach verified by autopsy. On comparison of this number with the clinically diagnosed cases we have an expression for the entirely numerical value of cancer of the stomach statistics based entirely on clinical diagnoses. Considerably greater importance, however, is attached to the information given by the various stages of the analysis — especially the evaluation of the clinical over- and underestimation — concerning the individual value of such statistics.

Cases with the Clinical Diagnosis »Cancer of the Stomach«. *Clinical Overestimation.*

Among the 8,633 cases covered by this investigation, 528 (6.1 %) were diagnosed prior to the autopsy as cancer of the stomach. It is

to be pointed out here that this rather high percentage does not apply to Danish material in general. Its great size is due, in part, to the fact that this investigation covers only the adults, partly to the circumstance that the investigation comprises only patients from surgical and medical departments, where cases of this kind accumulate. In addition to these 528 cases, the diagnosis cancer of the stomach was mentioned in 14 more cases but here merely as a subsidiary diagnosis — as, for instance, »peptic ulcer (cancer?)«, »cancer of the esophagus (cardia?)«, »cancer of the liver (stomach?)« — and these cases would not be included in cancer of the stomach statistics based on clinical diagnoses alone. On the other hand, all the first-mentioned 528 patients would most likely have been registered under cancer of the stomach if no autopsy had been performed, as this diagnosis was made either without any reservation whatever or at any rate as the most probable main diagnosis (see below).

Of the 528 patients who without autopsy would have been recorded in the stomach card index of the Cancer Register, 132 (25.0 %) proved *not* to be suffering from cancer of the stomach but from some other disease. *Thus only three-fourths (396 of 528) of the cases interpreted clinically as cancer of the stomach proved to have this lesion.* This is a considerable clinical overestimation.

The 132 fatal cases of other diseases taken to be cancer of the stomach may — as previously demonstrated in detail³ — be divided into two groups according to the certainty with which the clinicians thought this diagnosis could be made. In the first group of 92 cases cancer of the stomach is mentioned without any reservation and without any mention whatever of other possible diseases that may be mistaken for cancer of the stomach. So these 92 cases would certainly have been registered as cancer of the stomach if no autopsy had been performed.

The other group comprises 40 cases in which it was not considered safe categorically to make the diagnosis cancer of the stomach, as a question mark was affixed to the diagnosis, often with the addition of a symptomatic diagnosis as hematemesis, jaundice, etc. Among the diagnoses in these cases, however, other diseases that may occasion a mistake have been mentioned but exceptionally (5—6 times), and in these few cases cancer of the stomach is also put down as the first — *i. e.*, the most probable — clinical diagnosis. It will be reasonable, therefore, to assume that also this group of patients would be recorded under cancer of the stomach in a statistical account based merely on the clinical diagnosis. But as this may still be considered somewhat doubtful, it will be more appropriate to analyze the two groups separately.

The first group is analyzed in Table 1: The 92 fatal cases of other lesions erroneously interpreted clinically as cancer of the stomach which would certainly have been entered under this heading in a

statistical account based on clinical diagnoses. These cases are entered in Column I after sex and age at the time of death and compared with the clinically correctly diagnosed cases of cancer of the stomach, which are entered in Column II. Here the term »clinically correctly diagnosed cases« means cases which would have been entered in the cancer of the stomach statistics, also without autopsy, that is, cases diagnosed without any reservation as cancer of the stomach or — in 15 cases — with »cancer of the stomach (?)« as the only diagnosis. On calculation of the figures in Column I in percentage of the sum of Columns I and II (I + II) we find (as recorded in the last column) how great a part the cases erroneously taken as cancer of the stomach make of the total cases which without autopsy certainly would have

Table 4.

Fatal Cases which Clinically were interpreted Erroneously as Cancer of the Stomach and which, in the Absence of Autopsy, would have been included in the Cancer of the Stomach Statistics (I) as compared to the Cases of Cancer of the Stomach diagnosed Correctly in the Clinic (II).

Sex	Age	I	II	I + II	I in % of (I + II)
Men	20—29		1	1	
	30—39		5	5	
	40—49	7	27	34	20.6
	50—59	8	78	86	9.3
	60—69	31	82	113	27.4
	70—79	15	62	77	19.5
	80—89	2	6	8	25.0
	90—99				
	20—99	63	261	324	19.5 ± 4.4
Women	20—29		1	1	
	30—39		2	2	
	40—49	3	17	20	15.0
	50—59	7	22	29	24.2
	60—69	9	44	53	17.0
	70—79	5	40	45	11.1
	80—89	5	6	11	45.5
	90—99		2	2	
	?		1	1	
	20—99	29	135	164	17.7 ± 5.9
Men and Women	20—29		2	2	
	30—39		7	7	
	40—49	10	44	54	18.5
	50—59	15	100	115	13.0
	60—69	40	126	166	24.1
	70—79	20	102	122	16.4
	80—89	7	12	19	36.9
	90—99		2	2	
	?		1	1	
	20—99	92	396	488	18.9 ± 3.5

been entered in the cancer of the stomach statistics. After the main sums, twice the mean error of the calculated percentage is recorded.

From Table 1 it is evident that the clinical overestimation of cancer of the stomach (with sure diagnoses) is of about the same degree for men as for women, and that it is not particularly dependent on the age of the patient. Throughout this group it makes about one-fifth of the correctly diagnosed cases of cancer of the stomach.

In Table 2 the *second group* of fatal cases of other diseases erroneously taken clinically to be cancer of the stomach is analyzed, namely: the 40 patients who reasonably, though not with certainty, may be assumed to have been entered in the cancer of the stomach registration if no autopsy had been performed.

Table 2.

Fatal Cases Clinically interpreted Erroneously as Cancer of the Stomach, which reasonably may be assumed, in the Absence of Autopsy to have been included in the Cancer of the Stomach Statistics (I) as compared to the Cases of Cancer of the Stomach diagnosed Correctly in the Clinic (II).

Sex	Age	I	II	I+II	I in % of (I+II)
Men	20—29		1	1	
	30—39		5	5	
	40—49	1	27	28	3.6
	50—59	5	78	83	6.0
	60—69	7	82	89	7.9
	70—79	3	62	65	4.6
	80—89	2	6	8	25.0
	90—99				
	20—99	18	261	279	6.5 ± 3.0
Women	20—29		1	1	
	30—39	2	2	4	50.0
	40—49		17	17	
	50—59	5	22	27	18.5
	60—69	3	44	47	6.4
	70—79	10	40	50	20.0
	80—89	2	6	8	25.0
	90—99		2	2	
	?		1	1	
	20—99	22	135	157	14.0 ± 5.5
Men and Women	20—29		2	2	
	30—39	2	7	9	22.2
	40—49	1	44	45	2.2
	50—59	10	100	110	9.1
	60—69	10	126	136	7.4
	70—79	13	102	115	11.3
	80—89	4	12	16	25.0
	90—99		2	2	
	?		1	1	
	20—99	40	396	436	9.2 ± 2.8

According to Table 2, these cases make about one-tenth of the correctly diagnosed cases of cancer of the stomach. The grouping comprises comparatively more women than men (the difference is 2.2 times greater than the mean error, calculated with Yates' correction). In this group too there is no sure difference between the individual age-classes.

Table 3 gives the sum of the cases recorded in Tables 1 and 2.

Table 3.

All the Fatal Cases which Clinically were interpreted Erroneously as Cancer of the Stomach and which may be assumed, in the Absence of Autopsy to have been included in the Cancer of the Stomach Statistics — Clinical Over-estimation — (I) as compared to the Cases of Cancer of the Stomach diagnosed Correctly in the Clinic (II).

Sex	Age	I	II	I+II	I in % of (I+II)
Men	20—29		1	1	
	30—39		5	5	
	40—49	8	27	35	22.9
	50—59	13	78	91	14.3
	60—69	38	82	120	31.7
	70—79	18	62	80	22.5
	80—89	4	6	10	40.0
	90—99				
	20—99	81	261	342	23.7 ± 4.6
Women	20—29		1	1	
	30—39	2	2	4	50.0
	40—49	3	17	20	15.0
	50—59	12	22	34	35.3
	60—69	12	44	56	21.4
	70—79	15	40	55	27.3
	80—89	7	6	13	53.8
	90—99		2	2	
	?		1	1	
	20—99	51	135	186	27.4 ± 6.8
Men and Women	20—29		2	2	
	30—39	2	7	9	22.2
	40—49	11	44	55	20.0
	50—59	25	100	125	20.0
	60—69	50	126	176	28.4
	70—79	33	102	135	24.5
	80—89	11	12	23	47.8
	90—99		2	2	
	?		1	1	
	20—99	132	396	528	25.0 ± 3.8

The percentages recorded here may be used directly for the correction of cancer of the stomach statistics based on clinical diagnoses alone. By subtracting them from the total fatal cases with the clinical

diagnosis cancer of the stomach, we have the number of cases which on autopsy will prove to have been diagnosed correctly by the clinician.

*Clinically Non-recognized Cancer of the Stomach.
Clinical Underestimation.*

Among the 8,633 adult patients from surgical and medical departments, the presence of cancer of the stomach was demonstrated by autopsy in 596 cases (6.9 %). Of these cases, as mentioned before, 396 were clinically diagnosed correctly, that is, cases that would have been entered in the cancer of the stomach register also without autopsy. But in the remaining 200 cases (33.6 %) the diagnosis did not allow of such a recording of the patients. *Thus, without autopsy no less than one-third of all the cases of cancer of the stomach would have escaped registration.* This is the clinical underestimation.

The 200 clinically unrecognized cases of cancer of the stomach, of which a more thorough analysis has been given elsewhere² — among other things, with reference to the location of the tumor — fall into two groups: 1) the symptom-free cases (or at any rate with only slight symptoms), of which this material comprises 71, and 2) the symptom-giving cases in which the symptoms either were not judged correctly or erroneously mistaken to be brought about by some other disease, or cases in which the lesion was indicated merely by symptomatic or inexact diagnoses. Of these symptom-giving, erroneously diagnosed, cases of cancer of the stomach, the material comprises 129. Here, for the sake of brevity, we will designate these cases as diagnosed »erroneously«, even though many of them were diagnosed erroneously only in this sense that without autopsy they would not have been entered in the stomach index of the Cancer Register. For instance, diagnoses as cancer of the abdomen or cancer of the digestive tract are merely vague, not really erroneous, when involving cancer of the stomach, but their lack of precision makes them erroneous according to the point of view here adopted: whether or not the cases — on the basis of the clinical diagnosis — would be recorded as cancer of the stomach.

The 129 *erroneously diagnosed cases* of cancer of the stomach include all cases without the clinical diagnosis cancer of the stomach or the probability diagnosis »cancer of the stomach?« or one of their synonyms (a more thorough analysis of these diagnoses will be given in a subsequent paper⁴). The cases are entered after sex and age in Table 4, Column I, while here Column II gives the 396 cases of cancer of the stomach with correct clinical diagnosis. Column I + II shows the sums of the first two columns — that is, the total number of symptom-giving cases of cancer of the stomach. Finally, in the last column, the percentage of the cases with erroneous clinical diagnosis in relation to the ones with correct clinical diagnosis is calculated.

Table 4.

Number of Cases which Clinically were diagnosed Erroneously (I) and Cases of Cancer of the Stomach Clinically diagnosed Correctly (II), tabulated after Sex and Age of the Patients.

Sex	Age	I	II	I+II	I in % of II
Men	20-29		1	1	
	30-39	2	5	7	40.0
	40-49	11	27	38	40.8
	50-59	20	78	98	25.7
	60-69	24	82	106	29.3
	70-79	19	62	81	31.2
	80-89	3	6	9	50.0
	90-99				
	20-99	79	261	340	30.2 \pm 5.7
Women	20-29	2	1	3	200.0
	30-39	1	2	3	50.0
	40-49	3	17	20	17.6
	50-59	12	22	34	54.6
	60-69	18	44	62	40.9
	70-79	10	40	50	25.0
	80-89	4	6	10	66.7
	90-99		2	2	
	?		1	1	
	20-99	50	135	185	37.1 \pm 8.3
Men and Women	20-29	2	2	4	100.0
	30-39	3	7	10	42.8
	40-49	14	44	58	31.8
	50-59	32	100	132	32.0
	60-69	42	126	168	33.3
	70-79	29	102	131	28.7
	80-89	7	12	19	58.4
	90-99		2	2	
	?		1	1	
	20-99	129	396	525	32.6 \pm 4.7

In this way we are able from Table 4 directly to read the percentage which has to be added to the cases with correct clinical diagnosis verified by autopsy — which may be calculated from Table 3 on the basis of exclusively clinically diagnosed cases — in order to find the number of all the cases of symptom-giving cancer of the stomach demonstrable by autopsy.

Table 4 shows that the erroneously diagnosed cases occur with the same frequency in both sexes and that they are distributed about equally over the various age-classes. The number of cases with correct clinical diagnosis has to be increased by about one-third in order

to correspond to the total number of cases of symptom-giving cancer of the stomach demonstrable by autopsy.

In Table 5 the 71 *symptom-free* cases of cancer of the stomach or cases giving only slight symptoms are presented in a similar way (Column I) and, for comparison, the symptom-giving cases of the lesion ascertained on autopsy (Column II). Here the term »symptom-free« applies to alle the cases in which the clinical diagnosis does not mention cancer of the stomach or a synonym for this, or suspection of such a lesion, or a lesion that may be confused with cancer of the stomach, or a symptom suggestive of cancer of the stomach. All the patients here concerned have had other lesions figuring as the plausible cause of death, clinically as well as on autopsy (cf. the

Table 5.

Number of Symptom-free Cases (I) and Symptom-giving Cases (II) of Cancer of the Stomach ascertained on Autopsy, tabulated with reference to the Sex and Age of the Patients.

Sex	Age	I	II	I+II	I in % of II
Men	20—29		1	1	
	30—39		7	7	
	40—49	2	38	40	5.3
	50—59	5	98	103	5.1
	60—69	17	106	123	16.0
	70—79	14	81	95	17.3
	80—89	9	9	18	100.0
	90—99				
	20—99	47	340	387	13.8 ± 3.7
Women	20—29		3	3	
	30—39	1	3	4	33.3
	40—49		20	20	
	50—59	2	34	36	5.9
	60—69	3	62	65	4.8
	70—79	10	50	60	20.0
	80—89	7	10	17	70.0
	90—99	1	2	3	50.0
	?		1	1	
	20—99	24	185	209	13.0 ± 4.9
Men and Women	20—29		4	4	
	30—39	1	10	11	10.0
	40—49	2	58	60	3.5
	50—59	7	132	139	5.3
	60—69	20	168	188	11.9
	70—79	24	131	155	18.3
	80—89	16	19	35	84.2
	90—99	1	2	3	50.0
	?		1	1	
	20—99	71	525	596	13.5 ± 2.9

above-mentioned subsequent paper on further analysis of the diagnoses⁴).

Table 5 shows that the relative frequency of the symptom-free cases of cancer of the stomach is the same for men and women. In both sexes the frequency increases markedly with increasing age. About one-eight of the cases have been symptom-free or at any rate the symptoms have been so slight that no occasion was found in the diagnoses of these cases to mention any suspicion of cancer of the stomach or symptoms of this lesion.

The percentages calculated in the last column of Table 5 may be used directly for correction of the figures recorded in Table 4, as their addition gives the total number of symptom-giving as well as symptom-free cases of cancer of the stomach demonstrable on autopsy.

Discussion.

The 1069 autopsies which have been excluded from the present material because of the absence of clinical diagnoses would hardly have had any influence on the results here obtained. They include 43 cases of cancer of the stomach (4 %) which show a distribution as to the age and sex of the patients and the location of the tumor that is quite similar to that of the cases covered by this investigation². The frequency of the lesion in relation to the number of autopsies (4 %) is a little lower than the rate obtained for the autopsies here presented (6.9 %); and this is due to the circumstance that the excluded cases largely are made up of patients who died after a very short illness.

As this investigation goes as far back as to 1915 inclusive, the idea suggests itself that the improved diagnostic methods elaborated in the course of time may have brought about some chronological changes in the occurrence of the clinically non-recognized cases of cancer of the stomach as well as the fatal cases of other lesions which clinically were erroneously interpreted as cancer of the stomach. Table 6 shows the occurrence in the course of time of clinically non-

Table 6.

Number of Symptom-free (I) Cases of Cancer of the Stomach and Symptom-giving Cases (II) Clinically interpreted Erroneously as compared to all the Cases of this Lesion. Analysis of the Frequency in Brief Periods.

Period	I	II	III	I in % of III	II in % of III
1915—20	21	26	158	13.3	16.5
1921—25	17	32	151	11.3	21.2
1926—30	11	35	123	9.0	28.5
1931—35	22	36	164	13.4	22.0
1915—35	71	129	596	11.9	21.7

recognized cases of cancer of the stomach (symptom-free and diagnosed erroneously), *i. e.*, clinical underestimation.

From Table 6 it is evident that the clinically non-recognized cases of cancer of the stomach occur with uniform frequency throughout the period covered by this investigation.

In a similar way, Table 7 presents the clinical overestimation, *i. e.*, the cases of other diseases erroneously interpreted as cancer of the stomach.

Table 7.

Number of Fatal Cases of Other Diseases Clinically Interpreted Erroneously as Cancer of the Stomach. Some of these Cases (I) would certainly and others (II) probably have been included in Statistics based on Clinical Diagnoses Alone. Comparison of these Cases with the Number of Cases of Cancer of the Stomach diagnosed Correctly in the Clinic (III). Analysis of the Frequency in Brief Periods.

Period	I	II	III	I in % of III	II in % of III
1915—20	29	10	111	26.1	9.0
1921—25	19	10	102	18.6	9.8
1926—30	21	10	77	27.3	13.0
1931—35	23	10	106	21.7	9.4
1915—35	92	40	396	23.0	10.1

Like Table 6, Table 7 shows no chronological change whatever in the occurrence of the cases. Hence, it probably will be justifiable to consider the evaluation of the clinical diagnosis of cancer of the stomach resulting from this investigation as being valid generally till the introduction of diagnostic methods more effective than the ones commonly employed so far. In this connection it is to be mentioned that gastroscopy has been employed in the Kommune Hospital from the very first years covered by this investigation, but this examination has not been carried out in a majority of the cases which clinically were interpreted as cancer of the stomach.

For illustration of the length of time available for the clinical examination, it is to be mentioned that of the clinically non-recognized cases of cancer of the stomach as well as cases of other diseases erroneously taken to be cancer of the stomach, two-thirds were hospitalized for more than one week before exitus, and about one-half for more than two weeks.⁴ Of the cases clinically diagnosed correctly as cancer of the stomach, three-fourths were hospitalized for more than one week prior to exitus, and 50 % for more than two weeks.⁴ So, generally, no essentially longer period has been available for special examinations in the correctly diagnosed cases than in the erroneously diagnosed.

The nature of this investigation implies that the material may seem inadequate in two respects. In the first instance, this method of analysis is limited to patients who have died; in the second place

it is limited to hospitalized patients. Here where the investigation concerns cancer of the stomach, however, these limitations are of minor significance. The frequency of this lesion which in over 90 % of the cases terminates fatally in less than one year after the appearance of the symptoms, is about the same for each group of the living population as for the fatal cases within the corresponding age-classes, etc. As to the second limitation, a majority of the patients have at some time or other been staying in some hospital and hence a great majority of the cancer of the stomach patients in the population have been able to benefit from the complete diagnostic armamentarium of the hospitals and the special experience of the staff physicians. Even though only about 60 % of the cancer of the stomach patients in Copenhagen die in the hospitals,¹ there is no reason to distinguish between these 60 % and the other patients with this lesion as in a great majority of the cases the diagnoses have been made in the hospitals.

*Summary Autopsic Evaluation of the Clinical Diagnosis
»Cancer of the Stomach«.*

Through the preceding analyses we now have attained an approximately clear idea of how many cases of cancer of the stomach demonstrable on autopsy we may reckon with in a patient material where the diagnoses are entirely clinical. Table 8 presents the calculation in a simplified form. Here Column I gives the number of patients with the clinical diagnosis cancer of the stomach, and Column II gives the cases erroneously diagnosed as cancer of the stomach, while Column I—II presents the correctly diagnosed cases. The figures in Column II represent the clinical overestimation. Further, in Column III we have the clinically non-recognized cases of the lesion, that is, the clinical underestimation. Column I — II + III presents the number of cases ascertained on autopsy. Finally, the last column gives the percentage of this number in relation to the clinically diagnosed cases recorded in Column I. The percentages calculated in this way give directly the correction that has to be applied to the clinical diagnoses if these are to correspond to the occurrence of cancer of the stomach as ascertained by autopsy.

The result of Table 8 — and thus of the entire investigation — is, for one thing, that 12—13 % more cases of cancer of the stomach are ascertained by autopsy than may be recognized clinically; this clinical underestimation is particularly pronounced in the older age-classes. It quite corresponds to the occurrence of the symptom-free cases (cf. Table 5). If these cases be left out of the calculations — as shown in Table 9 — we find just as many cases of cancer of the stomach being diagnosed correctly in the clinic as are ascertained post mortem by autopsy, and their sex and age distribution is quite

Table 8.

Number of Cases diagnosed Clinically as Cancer of the Stomach (I), divided according to the Invalidation (II) or Confirmation (I—II) of the Diagnosis on Autopsy, as compared with the Clinically non-recognized Cases of this Lesion (III) and tabulated with reference to the Sex and Age of the Patients.

Sex	Age	I	II	I—II	III	I—II+III	(I—II+III) in % of I
Men	20—29	1		1		1	100.0
	30—39	5		5	2	7	140.0
	40—49	35	8	27	13	40	114.3
	50—59	91	13	78	25	103	113.2
	60—69	120	38	82	41	123	102.5
	70—79	80	18	62	33	95	118.8
	80—89	10	4	6	12	18	180.0
	90—99						
	20—99	342	81	261	126	387	113.2 ± 3.7
Women	20—29	1		1	2	3	300.0
	30—39	4	2	2	2	4	100.0
	40—49	20	3	17	3	20	100.0
	50—59	34	12	22	14	36	105.9
	60—69	56	12	44	21	65	116.1
	70—79	55	15	40	20	60	109.1
	80—89	13	7	6	11	17	130.8
	90—99	2		2	1	3	150.0
	?	1		1		1	100.0
	20—99	186	51	135	74	209	112.4 ± 4.9
Men and Women	20—29	2		2	2	4	200.0
	30—39	9	2	7	4	11	122.2
	40—49	55	11	44	16	60	109.1
	50—59	125	25	100	39	139	111.2
	60—69	176	50	126	62	188	106.9
	70—79	135	33	102	53	155	114.8
	80—89	23	11	12	23	35	152.1
	90—99	2		2	1	3	150.0
	?	1		1		1	100.0
	20—99	528	132	396	200	596	112.8 ± 2.9

corresponding hereto. But — and this is the other main result of this investigation — in one-fourth of the cases the individuals designated by the clinical diagnoses as cancer of the stomach patients are entirely different from the ones in whom the autopsy reveals the presence of cancer of the stomach.

Conclusion.

Cancer of the stomach statistics based on clinical diagnoses are encumbered with two shortcomings: In the first place, they do not include the ca. 12 % symptom-free cases (which is quite natural);

Table 9

Number of Cases Clinically diagnosed as Cancer of the Stomach (I) divided according to the Invalidation (II) or Confirmation (I—II) of the Diagnosis on Autopsy, as compared with the Clinically non-recognized Symptom-giving Cases of the lesion (III) tabulated with reference to the Sex and Age of the Patients.

Sex	Age	I	II	I—II	III	I—II+III	(I—II+III) in % of I
Men	20—29	1		1		1	100.0
	30—39	5		5	2	7	140.0
	40—49	35	8	27	11	38	108.6
	50—59	91	13	78	20	98	107.7
	60—69	120	38	82	24	106	88.4
	70—79	80	18	62	19	81	101.2
	80—89	10	4	6	3	9	90.0
	90—99						
	20—99	342	81	261	79	340	99.4 ± 0.4
Women	20—29	1		1	2	3	300.0
	30—39	4	2	2	1	3	75.0
	40—49	20	3	17	3	20	100.0
	50—59	34	12	22	12	34	100.0
	60—69	56	12	44	18	62	110.7
	70—79	55	15	40	10	50	90.9
	80—89	13	7	6	4	10	76.9
	90—99	2		2		2	100.0
	?	1		1		1	100.0
	20—99	186	51	135	50	185	99.4 ± 0.6
Men and Women	20—29	2		2	2	4	200.0
	30—39	9	2	7	3	10	111.1
	40—49	55	11	44	14	58	105.4
	50—59	125	25	100	32	132	105.6
	60—69	176	50	126	42	168	95.4
	70—79	135	33	102	29	131	97.1
	80—89	23	11	12	7	19	82.7
	90—99	2		2		2	100.0
	?	1		1		1	100.0
	20—99	528	132	396	129	525	99.4 ± 0.3

in the second place, they include only three-fourths of the symptom-giving patients. Numerically, however, the latter shortcoming is counterbalanced completely by the circumstance that the statistics include an equal number of individuals suffering from other lesions which clinically have been interpreted erroneously as cancer of the stomach. Furthermore, the age and sex distribution of these individuals is quite the same as that of the one-fourth of the cancer of the stomach patients missed by the clinical statistics. So the present cancer of the stomach statistics which largely are based on clinical diagnoses, have to be looked upon as numerically satisfactory, as

they are lacking merely the symptom-free cases of the lesion. But these statistics imply a very serious shortcoming as, to an essential extent, they include individuals other than cancer of the stomach patients.

Summary.

On the basis of 8,633 autopsies with the appertaining diagnoses made by the clinicians prior to the autopsy, a method is worked out empirically that enables us from statistics based on clinical diagnoses to calculate how many cases of cancer of the stomach demonstrable by autopsy are comprised by the patient material in question.

The symptom-free cases of the lesion make about 12 % of the total number.

Leaving these cases out of account, we find that the clinicians are able to diagnose just as many cases of cancer of the stomach as can be demonstrated by the pathologists, and the age and sex distribution of the patients is exactly the same as the one found for the autopsy material. But one-fourth of the cases in the clinical statistics involve individuals other than the ones who are suffering from this lesion. Numerically, then, the clinical over- and underestimation of symptom-giving cases counterbalance each other completely.

But we must realize that statistics based on the clinical diagnoses alone will to a considerable extent include patients other than those suffering from cancer of the stomach, while a corresponding number of patients with this lesion are missing in the clinical statistics.

Furthermore, in order to be numerically correct, the statistics are to be supplemented with the symptom-free cases of the lesion.

With the corrections calculated in this work, it will be practicable with fair proximation to give the frequency and age and sex distribution of fatal cancer of the stomach in the population numerically correct on the basis of the clinical diagnoses alone. But with our present diagnostic methods such statistics cannot be individually correct.

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A documentary survey of all cases forming the basis for the present work is left out here, as it has been published already in Folia cancerologica (Nos. 2 and 3 in this list of references).

3 NEW PNEUMOCOCCUS TYPES*)

By *Erna Mørch*.

(Received for publication May 19th, 1944).

According to the last communications from the State Serum Institute, Copenhagen, altogether 70 pneumococcus types have been established. As to these types, the reader is referred, for instance, to Kauffmann, Mørch & Schmith (1940) and Mørch (1942, 1943 and 1944).

In the State Serum Institute 3 additional new pneumococcus types have now been demonstrated; they are designated as: 15C, 28A and 35C. Owing to their close serological relation to types already known, the three new types are entered in previously established pneumococcus groups.

Type 15C.

Hitherto the pneumococcus group 15 consisted of Types 15 (Cooper and collaborators, 1932), 15A and 15B (Kauffmann, Mørch & Schmith). The formulas for the capsular antigens of these types were given by Kauffmann, Mørch & Schmith as follows:

$$\begin{aligned}\text{Type 15} &= 15a, 15b, 15c, 15f \\ \gg 15A &= 15a, 15c, 15d \\ \gg 15B &= 15a, 15b, 15e\end{aligned}$$

Antigen 15a is common to the 3 types, besides being found also in Type 23A (Table 8). Antigen 15b is common to Types 15 and 15 B. Antigen 15c is common to Types 15 and 15A. Antigen 15f, 15d and 15e are specific of the individual types.

The pneumococcus strain 5051/40 was isolated from the throat of a 2-year-old boy suffering from pneumonia. It was determined as belonging to the Type 15 group. But with the so-called »factor sera« (*i. e.*, sera absorbed with types within the group, whereas reactions

*) This work was carried out with the aid of a grant from the »P. Carl Petersen Fond«.

with types outside the group are not removed) the strain could not be identified with any of the known types within the group. It gave distinct capsular swelling with the factor serum 15c, which is specific of Type 15B, but not with serum 15b, 15f as would have been expected if it was a strain of Type 15B (Table 4).

Table 1.

Cross-reactions in Strains and Sera of Types 15C, 28A and 35C.

The figures in parantheses give the capsular titers.

Type	Marked		Cross-reactions with Types
15 C	5051/40	Strain	14 (16), 15 (40), 15A (320), 15B (160), 23 (2), 23A (16), 23B (4), 33 (8)
		Serum	15 (80), 15A (80), 15B (320), 23A (32)
28A	1800/43	Strain	16 (4), 23 (2), 23B (32), 24A (4), 28 (128), 39 (2)
		Serum	23B (32), 27 (16), 28 (128)
35C	5047/41	Strain	15A (2), 18 (2), 18B (32), 18 C (4), 20 (64), 29 (8), 31 (64), 33A (128), 34 (2), 35 (320), 35A (320), 35B (320), 42 (640)
		Serum	20 (64), 31 (64), 33A (128), 35 (128), 35A (640), 35B (640), 42 (320)

A serum produced by immunization of 5 rabbits gave cross reactions as shown in Table 1, where the heterologous reactions of the strain are recorded too. In both cases these reactions are examined for the remaining 72 pneumococcus types.

The strong reactions obtained with types within group 15 brought about that the strain examined was set up as a new type in the 15-group: Type 15C.

The new type (both the strain and the serum) reacts with Type 23A as do the remaining types of the group. Type 15C is able on

Table 2.

Results of Absorptive Tests for the Type 15 Group.

The figures give the capsular titers.

Strain	Serum 15				Serum 15 A				Serum 15 B				Serum 15 C			
	not abs.	abs. with			not abs.	abs. with			not abs.	abs. with			not abs.	abs. with		
		15A	15B	15C		15	15B	15C		15	15A	15C		15	15A	15B
15	160	128	64	128	80	0	8	8	160	0	32	32	80	0	0	0
15A	80	0	8	16	320	16	32	32	40	0	0	0	80	4	0	0
15B	160	64	0	64	160	4	0	0	640	> 256	> 256	> 256	320	64	64	0
15C	40	0	0	0	320	16	0	0	160	32	64	0	320	128	128	0

absorption of sera produced with Types 15, 15A and 15B to remove the reaction of these sera with Type 23A. Also the reactions of serum 23A with Types 15, 15A and 15B can be removed by absorption with Type 15C. So it must be the same antigen which the four types in the 15-group have in common with Type 23A, namely the previously established antigen 15a (Table 8).

Table 2 gives the results of cross-absorptions between Types 15, 15A, 15B and the new Type 15C, showing that Type 15C differs from Types 15, 15A and 15B. Type 15C is closely related to Type 15B, as serum 15C is emptied by absorption with Type 15B, whereas serum 15B is not emptied by Type 15C.

It will be appropriate here to present the establishment of the antigenic formulas for the types belonging to the 15-group. From Table 2 it will be noticed that all four types in this group react mutually and thus must have an antigen, 15a, in common. Further, it will be noticed that serum 15 absorbed with Type 15A reacts both with Type 15 and with Type 15B; so these two types must have a common antigen 15b. Serum 15 absorbed with Type 15B reacts with Types 15 and 15A, which thus must have an antigen in common, 15c. That serum 15 after absorption with Type 15C still reacts with Types 15, 15A and 15B can be explained as attributable to the factors 15b and 15c already established.

When serum 15A is absorbed with Type 15, it still reacts with Types 15A, 15B and 15C; these types must have an antigen in common, 15d. This antigen appears to be somewhat less developed in Type 15B, and on this account, antigen 15d was omitted in the previous antigenic formula for Type 15B (Kauffmann, Mörch & Schmith). As this antigen is present also in the new type, 15C, we have now recorded antigen 15d for Type 15B too in order to be able to explain the absorption results. Serum 15A absorbed with Type 15B or 15C reacts in either case with Types 15 and 15A, which can be explained by the presence of antigen 15c in these two types.

When serum 15B is absorbed with Type 15, one would expect that, owing to the factor 15d, this absorbed serum would react with Types 15A, 15B and 15C. The reaction with Type 15A is lacking, however, — something that possibly may be explained by inability of the rather poorly developed antigen

Table 3.

Results of Combined Absorptive Tests for the Type 15 Group.

The figures give the capsular titers.

Strain	Serum 15	Serum 15 A		Serum 15 B	Serum 15 C
	abs. with 15 A + 15 B	abs. with 15 + 15 A 15 + 15 C		abs. with 15 + 15 C	abs. with 15 + 15 A
15	64	0	0	0	0
15 A	0	2	2	0	0
15 B	0	0	0	> 256	32
15 C	0	0	0	0	64

15d in Type 15B to induce antibody formation in the serum 15B here employed. The reactions with Types 15B and 15C can be explained as attributable to the factor 15e, which will be dealt with below.

According to the formulas hitherto employed, serum 15B absorbed with Type 15A contains the factor 15h, which explains the reactions with Types 15 and 15B. But this serum reacts also with Type 15C — something that may be accounted for by assigning antigen 15e to both Type 15B and 15C. After absorption of serum 15B with Type 15C, the serum reacts, as expected, with Types 15 and 15B (antigen 15b).

The reactions of serum 15C after absorption with Type 15 or 15A are explained by the formulas already set up. As serum 15C on absorption with Type 15B is emptied of all antibody for the types in the 15-group, Type 15C cannot contain any specific antigen.

We shall now see whether Types 15, 15A and 15B might contain specific antigens. For this purpose, combined absorptions have been carried out as recorded in Table 3.

When serum 15 is absorbed with both Type 15A and Type 15B it still reacts with the homologous strain. Consequently, Type 15 must contain a specific antigen, 15f. Correspondingly, when serum 15A is absorbed either with Types 15 + 15B or with Types 15 + 15C, it still gives a very weak reaction with the homologous strain. Although this reaction is very weak, it still is included in the antigenic formula as 15g because it is specific of Type 15A.

Absorption of serum 15B with Types 15 and 15C leaves a strong homologous reaction. For this reason, Type 15B is set up with a specific factor, 15h. The reaction which serum 15C absorbed with Types 15 + 15A gives with Types 15B and 15C may be explained as attributable to factor 15e. As mentioned before, Type 15C contains no antigen specific of this type and hence it may be interpreted as a deficiency variant of Type 15B.

The formulas for the capsular antigens in the 15-group are given in Table 4. The antigens added to the previous formulas for Types 15A and 15B are italicized in the table.

The diagnosis within the group is made by means of the aforementioned »factor sera«. Table 4 gives the factor sera here employed which can be prepared as follows:

Serum	15b, f=	serum 15	absorbed with	Type	15A
"	15c, f=	" 15	"	"	15B
"	15d, g=	" 15 A	"	"	15
"	15e =	" 15 C	"	"	15A
"	15h =	" 15 B	"	"	15 + 15C

Distinction between the types within this group can be made by capsular swelling tests with these sera.

Diagnostic serum for the 15-group is produced by immunization with a mixture of Types 15, 15A, 15B and 15C. Type 15C may be omitted from this mixture as it contains no antigens specific of the type. The cross-reactions of this group serum are removed by absorption. A similar polyvalent, but not absorbed, group 15 serum is employed for therapy.

In mouse experiments Type 15C has proved to be but very slightly virulent.

Table 4.
Antigenic Formula and Factor Sera.

+ = positive capsular swelling.

— = no capsular swelling.

Type	Antigenic Formula	Factor Sera				
		15b, f	15c, f	15d, g	15 e	15 h
15	15 a, 15 b, 15 c, 15 f	+	+	—	—	—
15 A	15 a, 15 c, 15 d, 15 g	—	+	+	—	—
15 B	15 a, 15 b, 15 d, 15 e, 15 h	+	—	±	+	+
15 C	15 a, 15 d, 15 e	—	—	+	+	—
		28 b	28 c			
28	28 a, 28 b, 16 b, 23 d	+	—			
28 A	28 a, 28 c, 23 d	—	+			
		35 b, 34 b	35 c, 20 b	29 abs.	42 a	
35	35 a, 35 b, 34 b	+	—	—	—	
35 A	35 a, 35 c, 20 b, (35 d)	—	+	—	—	
35 B	35 a, 35 c, 29 b	—	+	+	—	
35 C	35 a, 35 c, 20 b, 42 a	—	+	—	+	

Type 28A.

Type 28 (Cooper and collaborators, 1932) is set up by Kauffmann, Mørch & Schmith with the antigenic formula:

Type 28 = 28a, 16b, 23d

28a is the antigen specific of the type. 16b signifies an antigen common to Type 28 and to Type 16. 23d is another antigen common to Type 28 and to Type 23B (Table 8).

The pneumococcus strain 1800/43 was isolated from the sputum of an 18-year-old man with bronchitis and bronchopneumonia. As the strain gave a rather slight capsular swelling with the diagnostic Type 28 serum, it was investigated more thoroughly.

A serum was produced by immunization of 5 rabbits. The cross-reactions for this serum and for the strain itself are given in Table 1. On account of the strong reaction with Type 28, cross-reactions were performed to see whether the new strain was identical with this type. The results are recorded in Table 5, showing that here we were

dealing with two different types. The new type was designated as 28A.

As Type 28 has antigens in common with Types 16 and 23B, it was natural to see whether this might apply to Type 28A too. The outcome of the cross-absorptions between these four types is shown in Table 5. From these experiments, then, it is practicable to set up an antigenic formula for Type 28A.

Table 5.

Results of Absorptive Tests for the Type 28 Group and the Types 16 and 23B.

The figures give the capsular titers.

Strain	Serum 28				Serum 28 A				Serum 16			Serum 23 B		
	not abs.	abs. with			not abs.	abs. with			not abs.	abs. with		not abs.	abs. with	
		28 A	16	23 B		28	16	23 B		28	28 A		28	28 A
28	320	64	128	>256	128	0	128	64	32	0	8	64	0	0
28 A	128	0	64	32	320	8	320	128	8	0	0	64	0	0
16	32	0	0	<2	0	0	0	0	640	640	320	0	0	0
23 B	64	0	16	0	32	0	16	0	0	0	0	640	160	160

The joint reactions of Types 28 and 28A must be due to a common antigen, 28a. Serum 28 absorbed with Type 28A reacts only with Type 28, not with Types 16 and 23B. Thus Type 28A must have emptied serum 28 of the factors 16b and 23d. The reaction with Type 28 must be due to an antigen, 28b, specific of this type. When serum 28A is absorbed with Type 28 it reacts only with Type 28A, which therefore must contain a specific antigen, 28c.

We will now see whether Type 28A contains antigens 16b and 23d. Serum produced by immunization with Type 16 reacts with Type 28A, whereas serum 28A gives no reaction with Type 16. Type 28A is able by absorption to remove the reaction with Type 16 from serum 28, whereas it is not able in spite of repeated absorptions to remove the reaction with Type 28 from serum 16. As the results thus are somewhat conflicting, and as it is a question merely of weak reactions, we have found it appropriate not to include 16b in the antigenic formula for Type 28A.

Type 28A contains antigen 23d — as is evident from the following experiment: both this strain, Type 28A, and the immune serum produced with it, react rather strongly with Type 23B (Tables 1 and 5). Type 28A is able by absorption to remove the reaction with Type 23B from serum 28, and also the reaction with Type 28 from serum 23B.

The antigenic formulas for the two types in the 28-group are given in Table 4. The diagnosis within the group is made with the factor sera 28b and 28c (Table 4).

Serum 28b = serum 28 absorbed with Type 28A
 „ 28c = „ 28A „ „ „ 28

Table 6.
Results of Absorptive Tests for the Type 35 Group and Type 42.
The figures give the capsular titers.

Strain	Serum 35			Serum 35 A			Serum 35 B			Serum 35 C			Serum 42		
	not abs.	abs. with 35A 35B 35C 43		not abs.	abs. with 35 35B 35C 42		not abs.	abs. with 35 35A 35C 42		not abs.	abs. with 35 35A 35B 42		not abs.	abs. with 35 35A 35B 35C	
35	640	128 16 32 340		160	0 0 0 64		320	0 0 (4) 320		80	0 0 0 63		0	0 0 0 0	
35 A	320	0 0 0 320		320	128 64 0 128		320	65 0 0 320		320	128 0 128 256		320	320 0 320 0	
35 B	320	0 0 0 320		160	16 0 0 128		640	128 64 32 320		160	32 0 0 128		16	16 0 0 0	
35 C	320	0 0 0 320		320	256 64 0 128		320	64 0 0 320		640	256 2 128 256		640	32 320 0	
42	0	0 0 0 0		160	160 64 0 0		2	(0) 0 0 0		160	128 2 128 0		1280	640 128 320 0	

Diagnostic and therapeutic group serum is produced in a manner corresponding to the one mentioned for the 15-group.

In mouse experiments Type 28A proved to be but slightly virulent.

Type 35C.

Hitherto the 35-group has comprised Types 35, 35A (Kauffmann, Morch & Schmith) and 35B (Morch, 1942 and 1943). The antigenic formulas for these types were set up as follows:

Type 35 = 35a, 35b, 34b
 „ 35A = 35a, 35c, 35d, 20b
 „ 35B = 35a, 35c, 29b

Antigen 35a is common to the three types. Antigen 35b is specific of Type 35, which in addition has a factor in common with Type 34, namely 34b (Table 8). Antigen 35c is found in both Types 35A and 35B. Besides, antigen 20b is found in Type 35A and also in Types 20, 31, 33A and 42. Antigen 35d, which is very weak, is common to Types 35A and 42. Antigen 29b is common to Types 35B and 29.

Pneumococcus strain 5047/41 was isolated from the mucous sputum of a 21-year-old man with bronchopneumonia. The strain gave capsular swelling both in group serum 35 and in type serum 42, on which account it was investigated more thoroughly.

An immune serum was produced by immunization of 5 rabbits. The cross-reactions for this serum and for the strain in question are recorded in Table 1. These cross-reactions indicated that the strain would be Type 35A or a type closely related hereto.

Then cross-absorptions were performed between Types 35, 35A, 35B, 42 and the new strain 5047/41, designated as Type 35C. The results are given in Table 6.

Table 7.
Absorptions with Regard to Antigen 20b.

The figures give the capsular titers.

Strain	Serum 35 C							Serum 20		Serum 31		Serum 33 A		Serum 35 A			Serum 42		
	not abs.	absorbed with						not abs.	abs. 35 C	not abs.	abs. 35 C	not abs.	abs. 35 C	not abs.	abs. 35 C	abs. 42	not abs.	abs. 35A	abs. 35C
20	32	0	0	<2	0	0	0	1280	320	64	<2	64	0	2	0	0	160	0	0
31	64	4	0	4	0	0	0	64	2	320	160	64	0	0	0	0	80	0	0
33A	128	16	8	0	0	0	0	128	0	64	0	640	160	2	0	0	160	0	0
35A	320	256	128	256	0	0	256	128	0	128	0	128	0	320	0	128	320	0	0
35C	640	256	128	256	2	0	256	64	0	64	0	128	0	320	0	128	640	32	0
42	160	64	64	64	2	0	0	64	0	64	0	128	0	160	0	0	1280	128	0

From Table 6 it is evident that Type 35C differs from Type 35A, but the difference is only slight. Serum 35A is emptied by absorption with Type 35C; but, when serum 35C is absorbed with Type 35A, the remaining rest reacts with Types 35 C and 42. Furthermore, Type 35C is able to empty a serum produced by Type 42 — something which Type 35A is not able to do. Absorption of serum 35C with Type 42 leaves a serum which reacts strongly with Types 35, 35A, 35B and 35C. The antigenic formulas set up for the types in the 35-group and for Type 42 are given in Tables 4 and 8.

These antigenic formulas are set up on the basis of experiments in Table 6. The formulas for Types 35, 35A, 35B and 42 will not be mentioned in detail here (cf. previous works by the writer). It is to be mentioned, however, that in Types 35A and 42 we have chosen to disregard the weak antigen 35d. Likewise, in Types 29, 33A and 42 we have omitted the weak antigens 29c and 33g (Table 8). It is for the sake of a more easy survey of the formulas that we decided here to disregard these antigens which on further investigation proved to be developed but weakly. In setting up the antigenic formulas we try as far as possible to maintain the principle only to include the strongest antigens in the formulas together with the ones that are of importance to the diagnosis.

The antigenic formula for Type 35C is set up as follows: When Type 35C, as mentioned, by absorption is able to empty the serum produced by Type 35A, it must contain the same antigens as this type, namely 35a, 35c, 20b. But, as serum 35C is not emptied on absorption with Type 35A, then Type 35C must contain at least one antigen more than Type 35A. As, furthermore, serum 42 is emptied on absorption with Type 35C, the latter must contain the same antigens as are present in Type 42. But serum 35C absorbed with Type 42 is still able to react with the types in the 35-group, and hence Type 35C must contain at least one antigen that is not present in Types 42. When we set up the following antigenic formula for Type 35C we are able to explain the results recorded in Table 6 (with the exception of one reaction with low titer, which here is put in brackets).

Type 35C = 35a, 35c, 20b, 42a

The following experiment shows that antigen 35c is common to Types 35A, 35B, 35C and 42. Serum 35A is absorbed with both Types 20 and 35, which remove the factors 20b and 35a, leaving a serum containing the factor 35c. This serum reacts with the types mentioned: 35A, 35B, 35C and 42.

Through absorption experiments it has been demonstrated that antigen 20b is identical in Types 35A, 35C and 42, while this antigen differs a little from antigen 20b in Types 20, 31 and 33A (Table 7). No attempt has been made to give a thorough account of this fact (cf. Morch, 1943, Table 53).

The diagnosis within the 35-group is made by means of the factor sera employed hitherto (Morch, 1943):

Serum 35b, 34 b	= serum 35	absorbed with Type 35A.
" 35c, 20b	= " 35A	" " " 35
" 29 absorbed	= " 29	" " " 35+35A.

Table 8.

Schema of the Capsular Antigens of the Pneumococci (73 Types).
(After *Kauffmann, Morch & Schmith* and *Morch*, 1944).

* revised or new formulas.

Type	Antigenic formulas	Type	Antigenic formulas
1	1a	19C	19a, 19c, 19f, 7h
2	2a	20	20a, 20b, 7g
3	3a	21	21a
4	4a	22	22a, 22b
5	5a	22A	22a, 22c
6A	6a, 6b	23	23a, 23b, 18b
6B	6a, 6c	23A	23a, 23c, 15a
7	7a, 7b	23B	23a, 23b, 23d
7A	7a, 7b, 7c,	24	24a, 24b, 24d, 7h
7B	7a, 7d, 7c, 7h	24A	24a, 24c, 24d
7C	7a, 7d, 7f, 7g, 7h	24B	24a, 24b, 24c, 7h
8	8a	25	25a, 25b
9A	9a, 9c, 9d	27	27a, 27b
9L	9a, 9b, 9c, 9f	*28	28a, 28b, 16b, 23d
9N	9a, 9b, 9c	*28A	28a, 28c, 23d
9V	9a, 9c, 9d, 9g	*29	29a, 29b, 13b, (29c)
10	10a, 10b	31	31a, 20b
10A	10a, 10c, 10d	32	32a, 27b
11	11a, 11b, 11c	32A	32a, 32b, 27b
11A	11a, 11c, 11d, 11c	33	33a, 33b, 33d
11B	11a, 11b, 11f	*33A	33a, 33b, 33d, 20b, (33g)
12	12a	33B	33a, 33c, 33d, 33f
13	13a, 13b	33C	33a, 33c, 33e
14	14a	34	34a, 34b
15	15a, 15b, 15c, 15f	35	35a, 35b, 34b
*15A	15a, 15c, 15d, 15g	*35A	35a, 35c, 20b, (35d)
*15B	15a, 15b, 15d, 15c, 15h	35B	35a, 35c, 29b
*15C	15a, 15d, 15e	*35C	35a, 35c, 20b, 42a
16	16a, 16b, 11d	36	36a, 9c
17	17a	37	37a
18	18a, 18b, 18c, 18f	38	38a, 25b
18A	18a, 18b, 18d	39	39a, 10d
18B	18a, 18b, 18e, 18g	40	40a, 7g, 7h
18C	18a, 18b, 18c, 18e	41	41a
19	19a, 19b, 19d	*42	42a, 20b, 35c, (29c, 33g, 35d)
19A	19a, 19c, 19d	43	43a
19B	19a, 19c, 19e, 7h		

The serum employed for the diagnosis of Type 42 cannot be made type specific, as removal of the reaction with Type 35C would empty the serum of antibodies. Hence the diagnostic serum 42 reacts with Type 35C. In addition, this type also reacts with the diagnostic serum for the 35-group. So, if we find that a pneumococcus strain gives reaction both in the serum for the 35-group and in type serum 42, we further examine the strain with a serum 35C that has been absorbed with Type 42. This serum contains an ample amount of antibody for antigen 35a, and hence it reacts with all the types in the 35-group, but not with Type 42. If a reaction is obtained in this serum the strain must be a Type 35C, as Types 35, 35A and 35B give no capsular swelling in the diagnostic serum 42 here employed. For additional control, another test may be made with employment of a factor serum 42a (Table 4). This serum reacts within the 35-group only with Type 35C.

Serum 42a = serum 42 absorbed with Type 35A.

One might perhaps feel tempted to enter Type 42 in the 35-group on account of its close relationship with Types 35A and 35C, but we have refrained from this because Type 42 has no antigen in common with all types in the 35-group.

Kauffmann & Langvad-Nielsen have demonstrated that pneumococcus sera produced with the types belonging to the 35-group (35, 35A, 35B) agglutinate *Salmonella kirkee* strongly. As serum produced with Type 35C likewise agglutinates *Salmonella kirkee* (agglutinative titer 80), this reaction confirms that the type belongs to the 35-group. Serum 42 does not agglutinate the *Salmonella* strain mentioned.

In mouse experiments, Type 35C proved to have a very slight virulence. For the sake of comparison it may be mentioned that the serologically near-related Types 35A and 42 likewise are only slightly virulent for mice (Mørch, 1943).

Summary.

3 new pneumococcus types are set up, namely: 15C, 28A and 35C. On account of their serological relationship with types already known, the three new types are entered in pneumococcus groups established previously.

Formulas are given for the antigens of these types.

Now altogether 73 different pneumococcus types are known, distributed on 15 type groups and 26 single types.

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METHOD OF PRODUCING PURIFIED AND CONCENTRATED STAPHYLOCOCCIC TOXOID

By *Arne Lithander.*

(Received for publication May 31st 1944).

As soon as attempts to produce staphylococcic toxin began to prove successful, several workers set about preparing staphylococcic toxoid. Most of them treated the toxin with formalin and heat according to the principles which Ramon followed for the production of diphtheria toxoid. Ramon and his co-workers also made a number of valuable observations when they attempted to produce staphylococcic toxoid. They used staphylococcic toxin produced on different media and from different strains. But it proved to be more difficult to produce staphylococcic than diphtheria toxoid. Thus they observed a considerable loss of the antigenic value after the detoxication of the toxin with formalin and heat. The losses varied according to the method used for producing the toxin, and also according to the staphylococcic strain. Research of Ramon (1939) and Ramon and Richou (1940) showed that the antigenic losses on the detoxication of toxin produced from certain strains amounted to 12 to 15 per cent. In the case of toxins prepared from the Wood strain and others, on the other hand, the greater part of the antigenic value disappeared. This happened regardless of how much formalin was used, of whether the formalin was added in one or several lots, and of variations in the temperature. After 7 to 8 days in the thermostat the antigenic value was not greatly reduced, but the detoxication was not complete. After 13 or 14 days the detoxication was complete but the greater part of the antigen destroyed. When synthetic media were used the losses were smaller than with media like Martin bouillon, broth prepared from calf spleen according to Ramon, Berthelot and Amoreux (1936) and media made from pig's stomach. Toxins of several other strains prepared with the last-mentioned medium lost the bulk of their antigenic value during detoxication.

Only a few strains are suitable for the preparation of toxin. Most authors have prepared highly potent toxins from the Wood strain.

But attempts to prepare toxoid from these toxins have not proved successful hitherto and a new method seems to be needed. In the present investigation toxins prepared from the Wood strain according to my method (1944) were used for the production of toxoid. Judging from the aforementioned research, the period of time allotted to the detoxication is of great importance. Since the antigenic loss is considerably less after 7 or 8 days than after 14, it may be assumed that reduction of the time to less than 7 days would mean less loss of antigen. More rapid detoxication requires an increase in the amount of formalin added. An increase in the formalin has the disadvantage, however, that when the detoxication is completed, the unconsumed formalin remains and the antigen continues to undergo destruction even when the detoxicated product is removed from the thermostat and placed in a cold room. It is necessary, therefore, to remove the excess formalin immediately after the detoxication is complete. I have attempted to do this by saturating the crude toxoid with ammonium sulphate so that the antigen is precipitated and freed from the excess formalin. This procedure also purifies the toxoid. Purification of staphylococcic toxin by precipitation with ammonium sulphate was mentioned by Holt (1936).

It was first necessary to determine the formalin content and temperature which caused the smallest loss of antigen during the detoxication. Temperatures of 37, 39 and 40 C. were compared and 37 C. found to be the best. Formalin was tested in amounts of 4, 5, 6, 8 and 10 cc. per liter.

The strength of the toxins and toxoids was titrated against standard serum in the ordinary way according to the following methods. In the first case decreasing amounts of toxin were added to a series of tubes, each containing 1 antitoxin unit, and then physiologic salt solution to bring the volume to 2 cc. After thirty minutes at room temperature 0.5 cc. of a 2 per cent suspension of washed rabbit erythrocytes was added. Readings were made after the tubes had stood for one hour in the thermostat and one hour in room temperature. In the tubes where the toxin was not completely neutralized by the added antitoxin, the excess toxin caused hemolysis of the erythrocytes. The Lh value of the toxin was calculated from the smallest amount causing a trace of hemolysis.

The toxoid was titrated in the following manner. To each of a series of tubes each containing one unit of antitoxin in a volume of 1 cc. was added decreasing amounts of toxoid, and the volume made up to 2 cc. with physiologic salt solution. After thirty minutes in room temperature a fixed amount of a toxin of known strength was added to each tube. After another thirty minutes at room temperature 0.5 cc. of a 2 per cent suspension of washed rabbit erythrocytes was added to each tube. Readings were made after the tubes had stood one hour in the thermostat and one hour at room temperature. The smallest amount of toxoid together with which the toxin in a tube produced a trace of hemolysis was then taken as a basis for the calculations. In this tube approximate equilibrium existed between antigen and antitoxin. If it is assumed that the tube contained a cc. of toxin and b cc. of toxoid, it follows that 1 unit of antitoxin = a cc. of toxin

+ b cc. of toxoid. If it is further assumed that the Lh dose of the toxin used is a_1 cc. and that the antigenic value of the toxoid per cubic centimeter is x units it follows that

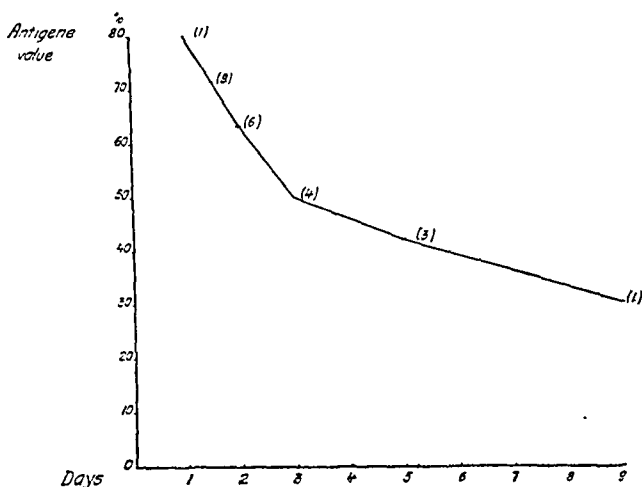
$$1 = \frac{a}{a_1} + bx$$

It follows from this equation that

$$bx = \frac{a_1 - a}{a_1} \text{ and}$$

$$x = \frac{a_1 - a}{ba_1}$$

It was found that with 5 and 4 cc. of formalin per liter the detoxication was complete after 2 and 3 weeks, respectively, and that most of the antigen was then lost. When 8 and 10 cc. per liter was used, the detoxication was complete after about 24 hours at the price of considerable loss of antigen. The most suitable amount of formalin proved to be 6 cc. per liter. With this concentration the detoxication was generally complete within 48 hours. Sometimes more time was needed, in one case nine days. The attached graph shows how the length of time taken for detoxication influenced the amount of antigen in the toxoid.



The relation between the time taken for detoxication and the antigenic value remaining after detoxication. The antigenic value in the crude toxoid in percentage of the value in the respective toxin is given on the ordinate. The time in days is plotted on the abscissa. Each antigenic value marked on the curve is the average of observations the number of which is shown in brackets beside.

The figure shows that the antigenic losses increased with the length of time taken by the detoxication. When, as in one case, the detoxication was complete in 24 hours, there was only a 20 per cent loss in antigen. Favorable results of this kind can only be expected in ex-

ceptional cases. In many cases, however, the detoxication was complete after 36 hours and the antigenic loss was then only slightly greater.

As mentioned, it is important to remove the unconsumed formalin from the toxoid immediately full detoxication has taken place. In the present experiments the excess formalin was rapidly removed in the following manner. The toxoid was taken out of the thermostat two days after the addition of formalin. Immediately afterwards it was tested that the toxoid did not hemolyze washed rabbit erythrocytes. As soon as this was done, 72 Gm. of dried ammonium sulphate per 100 cc. of toxoid was added. A yellowish brown substance often of a tough, sticky consistency, was then precipitated. Most of it floated up to the surface, and a small amount fastened on the walls of the vessels. When the mixture was stirred with a glass rod, the substance floating on the surface fastened to it and was easily removed. This part of the precipitate as well as that which fastened on the walls of the vessel was washed with a saturated solution of ammonium sulphate so as to remove undissolved sulphate and excess formalin. All of the precipitate was dissolved in a suitable amount of distilled water, usually one-quarter the volume of the crude toxoid. The dissolved precipitate was then dialysed against water or an 0.05 per cent solution of sodium bicarbonate until the sulphate disappeared completely, which generally happened after four or five days. When the dialysis was completed merthiolate was added to a concentration of 0.01 per cent and the solution filtered through paper. The purified toxoid so produced was a clear fluid with a brownish yellow color. On testing of the purity it was found that the purified toxoid contained 16 to 28 units per milligram of nitrogen while the crude toxoid contained only 2.5 to 3.8 units per milligram.

Precipitation with ammonium sulphate often causes loss of antigen. The loss, which amounts to 10 or 20 per cent of the crude toxoid's value, is partly due to lack of caution in collecting the precipitate. Sometimes there is a loss during the dialysis amounting to at the most 10 per cent of the value for the toxoid solution before the dialysis. The antigenic value of the prepared, purified toxoid amounts on the average to 56.5 per cent of that of the original toxin.

The purified toxoid was then tested for strength and nontoxicity. The strength was tested by titration *in vitro* according to the aforescribed method and by examination of its immunizing capacity in guinea pigs. The nontoxicity was tested by checking that it did not hemolyze washed rabbit erythrocytes and by tests on animals. As regards the latter, 0.2 cc. of undiluted toxoid injected intracutaneously into rabbits was only allowed to cause pale reddening, no necrosis. When injected intravenously into two rabbits in a dose of 2.5 cc. per kilogram of body weight, it was not allowed to cause death within three days.

When the toxoid was injected into rabbits, antitoxin formation

was observed both after intravenous and subcutaneous administration. The values varied between 2.5 and 33 units per cubic centimeter. When kept at 4 C. the antigenic value of the toxoid was unchanged after three months. After six months it was reduced 10 to 20 per cent. Storage in the frozen state increased the stability.

Staphylococcic toxoid has been used successfully for therapeutic purposes by, among others, Ramon and his co-workers and Thieffry. In order to gain an idea of the therapeutic value of the purified and concentrated toxoid described herein, it was tested in cases of human staphylococcic infection, such as furunculosis, sycosis staphylogenes and other conditions. The results of these investigations will be published in a later paper.

Summary.

Purified and concentrated staphylococcic toxoid was prepared from staphylococcic toxin made with the Wood strain by allowing a relatively high concentration of formalin to act on the toxin during warmth for a short time. Immediately detoxication had taken place, the toxoid was precipitated with ammonium sulphate. The precipitate was dissolved and dialyzed.

On the average 56.5 per cent of the antigenic value of the original toxin remained in the purified toxoid.

The toxoid caused the formation of antitoxin when injected into rabbits.

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METHOD OF PRODUCING STAPHYLOCOCCIC TOXIN

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(Received for publication May 31st 1944).

The tragedy in Bundaberg in 1928, when 19 out of 21 children died from intoxication after the injection of a mixture of diphtheria toxin and antitoxin contaminated with staphylococci, led to intensive studies on the toxins of staphylococci. During the years that followed many different kinds of culture media were elaborated for the production of staphylococcic toxin. It was soon realized that it was important to add carbon dioxide to the atmosphere in which the staphylococci were grown for the production of toxin. Burnet (1930 and 1931), in particular, drew attention to this detail. It was also found that more potent toxin was formed when agar was added to the media to make them semisolid. Since Dolman's report of this fact, most workers have added agar. Dolman (1935) himself obtained good toxins with media of this kind. Ramon (1936 and 1939) had good results with media resembling those he used for the production of diphtheria toxin, such as Martin broth and media prepared from pig's stomach. Ramon, Berthelot and Amoreux (1936) described a medium made from calf spleen with which they obtained highly potent toxins. Ramon, Boivin and Richou (1938) prepared toxins with values up to 40 units with synthetic media. Casman (1940) described a medium containing agar, with which he obtained toxins with more than 100 units. This seems to be the highest value reported in the literature.

The research on which the present article is based was done with the intention of producing a staphylococcic toxin with a high titer of alpha hemolysins suitable for the production of anatoxin. Attempts with Casman's method gave only 10 units per cubic centimeter. Strain Wood 46 was used, and so the poor results can hardly be ascribed to an unsuitable strain.

To get better results certain modifications were made in Casman's method. Fernbach flasks were used. Two holes were cut out opposite one another in their walls and glass tubes fused into them. Cotton plugs were inserted into the external openings of the tubes. The flasks were then coupled to one another in series by means of rubber tubes

attached to the glass ones, and connected with a container filled with a suitable gas mixture under pressure. From this container the gas mixture could be led through twelve to fifteen flasks. As a rule 25 per cent carbon dioxide and 75 per cent air was used.

I used a modified form of the medium described by Casman. Minced veal, 500 grams per liter of water, was extracted in distilled water at 80 to 90 C. for two hours and then filtered through gauze and paper. Difco peptone, 20 grams per liter, and sodium acetate, 7 grams per liter, were added and the pH was adjusted to 6.8. The mixture was sterilized in an autoclave. Immediately before the medium was used, the mixture was warmed to about 55 C. and 5 per cent melted agar was added to a suitable concentration. The medium was then put into Fernbach flasks treated in the way previously described and rinsed before the sterilization with diluted sodium hydroxide, hydrochloric acid and distilled water.

Strain Wood 46, which produces mostly alpha toxin, was used in the experiments. Before use it was passed through rabbits. About 24 hours after an intravenous injection of the strain, when the rabbit was moribund, the heart blood was placed in bouillon tubes. The cultures so obtained were centrifuged and the broth removed. A thick suspension of the bottom layer of the broth cultures was then seeded on the medium in the flasks. Immediately afterwards the flasks were placed in a thermostat and the gas mixture allowed to stream through them.

The factors which may be thought to have the greatest influence on the toxin production are the concentration of agar in the medium, the amount of medium in each flask and the period of time elapsing between the seeding and the harvest.

Generally 0.5 and 0.3 per cent concentrations of agar were used. Testing of higher and lower concentrations, 1 and 0.2 per cent respectively, proved them to be inferior. Comparison showed that 0.5 per cent gave better results than 0.3 per cent in most cases. This fact was assumed to make up for the reduction in the amount of culture medium when 0.5 per cent agar was used.

If the amount of medium in the flasks is varied, the relationship between the free surface and volume of the medium is also varied. In addition different amounts of medium mean differences in the time necessary for the bacterial growth to reach the different parts of the medium. Comparisons were made with 100, 200 and 300 cc. per flask. It was found that 300 cc. per flask gave a much weaker toxin than 100 or 200 cc. No definite difference could be seen between the last two amounts.

In order to determine the importance of the time factor, the results were compared after 24, 48, 72 and 96 hours in the thermostat. The best growth was seen after 48 hours. Slightly worse results were obtained after 72 hours, and 24 and 96 hours proved to be unsuitable.

When the flasks were removed from the thermostat the cultures were centrifuged, resulting in a bottom layer consisting mostly of agar and a clear light yellow fluid above. The supernatant fluid containing the toxin was pipetted off and the bottom layer discarded. The fluid was passed through paper and Seitz filters. Merthiolate was then added to a concentration of 1:10,000. Checks on the sterility about two days later generally showed no living staphylococci in the toxin.

The strength of the toxin was estimated from its capacity to hemolyze rabbit erythrocytes washed with physiologic salt solution. A 2 per cent suspension of washed corpuscles was used. The direct titration was done as follows. Decreasing amounts of toxin were pipetted into Widal tubes and the volume made up to 1.5 cc. with salt solution. After the addition of 0.5 cc. of the suspension of blood corpuscles the tubes were placed in a thermostat for one hour. The amount of toxin which produced complete hemolysis of the corpuscles and the amount which cause a trace of hemolysis were then noted. The Lh values were calculated from readings after one hour's incubation and after one hour at room temperature. The dilution of toxin showing 20 per cent hemolysis was taken as the end point.

This method, with an agar concentration of 0.5 per cent, 200 cc. of medium in each flask, a gas mixture of 25 per cent carbon dioxide and 75 per cent air and a growth period of 48 hours generally gave good toxins. However, the quality of the toxin seems to be highly dependent on other factors than the use of a good toxin-producing strain. The same strain does not always give the same good toxin. Its toxin-forming capacity seems to be intimately connected with the rabbit passages. If the strain shows poor toxin-forming capacity after a certain rabbit passage, the capacity usually does not improve after further passages.

As a rule the toxins had Lh values varying between 16.7 and 33.3 (12.5 and 66.7) units. The smallest amount which caused complete hemolysis of 0.5 cc. of a 2 per cent suspension of washed rabbit erythrocytes was 0.0007 to 0.002 cc., while 0.00001 to 0.0001 cc. were required for a trace (10 per cent) of hemolysis. When injected intravenously into rabbits, 0.02 to 0.05 cc. of the toxin per kilogram of body weight generally killed the animals within ten minutes. Distinct dermonecrosis (5 by 5 mm.) was produced on the intracutaneous injection of 0.00003 to 0.0002 cc. of most of the toxins.

Storage at + 4 C. weakened the toxin, the smallest amount causing hemolysis and the Lh dose then rising. After six months' storage the Lh dose increased between 30 and 50 per cent. After one month's storage at 4 C. no definite weakening was observed. When frozen the toxin retained its strength for over six months.

Summary.

The author describes a method of producing staphylococcic toxin on an agar-containing medium and in an atmosphere containing added carbon dioxide.

The toxin so produced hemolyzed rabbit blood corpuscles, when intracutaneously injected into rabbits caused dermonecrosis and when intravenously injected, even in small doses, caused rapid death of the animals.

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BACTERIAL CONTAMINATION OF BLOOD SAMPLES AS A SOURCE OF ERROR IN ANTISTREPTOLYSIN TITRATION

By *Sven Löfgren*.

(Received for publication June 20th 1944).

The antistreptolysin reaction according to *Todd* has proved to be a valuable serological test for demonstrating antibodies against hemolytic streptococci. In 1942, *Kalbak* made a detailed survey of the antistreptolysin investigations made up to that year and dealing particularly with rheumatic fever. In 1943, I published a preliminary report of the results obtained from studies on antistreptolysin in erythema nodosum.

In my investigations, the strength of the streptolysin was determined against a standard serum which had been kindly supplied by *Todd* and by *Kalbak*. Thus, I titrated the volume of streptolysin which was just neutralized by one unit of antistreptolysin. This amount contains one unit of streptolysin. The antistreptolysin content in an unknown serum was then determined by titrating the quantity of serum needed to neutralize the streptolysin unit. Most investigators have used a fairly complicated method for this, making two titrations on each sample. In some cases, also, the series of serum dilutions has been quite irregular, with an increase in dilution varying between 10 and 100 per cent. For my experiments I devised a simplified procedure, the following conditions being observed:

- 1) The serum dilutions should follow a geometrical series.
- 2) The interval chosen between the different dilutions should permit a fairly exact determination to be made.
- 3) The values in the so-called normal range, 0—100 units, should be determined as exactly as the other values.
- 4) The titration should be technically simple and capable of being used for routine purposes.

A series with a quotient of $4/3$ was chosen, this giving a logarithmic increase of 0.125. This type of series shows a strange mathematical feature which enhances its value. $\text{Log } 4/3 = 0.125$. $8 \times 0.125 = 1$. Thus $(\frac{4}{3})^8 = 10$. This means that the series is periodic, with 8 terms in each period. A serum dilution of 1:12 was chosen as the starting dilution, this giving the following series: 12 16 21 28 38 51 67 90 120 160 210 280, and so on.

The serum dilutions constituting the inverted values of these figures were obtained by the following method.

Amounts of 0.5 ml. of normal saline were placed in a series of test tubes, all except for the first tube. To the first tube 0.5 ml. of the desired serum dilution, in this case 1:6, was added and to the second tube 1.5 ml. After mixing, 1.5 ml. was transferred from the second to the third tube, and so on. As has already been stated, the starting serum dilution was to be 1:12. As it was necessary, however, for technical reasons, to work with 0.5 ml. of serum dilution in the tubes instead of with 1 ml. as recommended by Todd, the serum in the first tube was diluted only 1:6. To compensate for this, the streptolysin was diluted to double the quantity used in earlier techniques, 1 ml. of solution thus containing 1 unit of streptolysin. After 1 ml. of streptolysin solution had been added to each tube the total amount of serum and streptolysin was 1.5 ml. as it was in Todd's experiments. As the volume of antistreptolysin was calculated per ml. of serum, the serum dilution in the first tube thus corresponded to 1:12 with this procedure.

A 5 per cent suspension of blood corpuscles from sheep was used as indicator in these experiments. For further details as to method the reader is referred to the publications of Todd.

The antistreptolysin value in a titrated sample of serum was obtained by reading off the highest serum dilution which would still cause inhibition of the hemolysis. The inverted value of the dilution figure in this tube was equal to the number of antistreptolysin units in 1 ml. of serum. Thus, with this method, the antistreptolysin value is obtained by a direct reading, and there is no necessity to make further calculations.

As regards the technique for drawing the blood samples and preserving the serum, some authors (*Coburn and Pauli; Kalbak*) maintain that the samples should be kept under sterile conditions. No particular stress is laid on this point, however, and above all no attention has been paid in the literature to the consequences which may arise if the samples of blood have become contaminated. The present paper will show how bacterial contamination of the serum, especially contamination with *Pseudomonas* bacteria, can cause an excessive rise in the antistreptolysin titre.

During the course of my studies I had sometimes observed that hemolyzed samples of blood occasionally gave unaccountably high antistreptolysin values. On one occasion it happened that some samples were kept for a few days at the hospital at which they had been taken, before being sent to the laboratory for analysis. When they were finally examined, a couple of samples were hemolyzed and yielded titration values of 2000—3000 units while the patients concerned

had proved to have a titre of about 50 units when samples had been taken on earlier occasions. A control showed that the hemolysis as such could not have been the explanation of the high titre. I therefore suspected that both the hemolysis and the abnormal antistreptolysin value might be due to contamination of the samples. Cultures from both the samples yielded growths of *Pseudomonas fluorescens*. The two strains will be called in the remainder of this paper Fl. 1 and Fl. 2. When normal serum was seeded with these strains a marked rise in the antistreptolysin titre was produced within a short time. This feature is shown in the appended tables. Samples of the same serum were also inoculated with two strains of the closely allied *Pseudomonas pyocyanea*, designated below as P. 1 and P. 2. The effect was similar in these tests. Tables 1—3 show the variations in the antistreptolysin titre (AST) in different samples of serum, contaminated with Fl. 1, Fl. 2, P. 1 and P. 2, after a varying number of days and after the samples had been kept at different temperatures (in Celsius).

Table 1.

Serum sample 2742. AST before contamination = 38 units.

Titration after	Fl. 1		Fl. 2		P. 1		P. 2	
	+20°	+6°	+20°	+6°	+20°	+6°	+20°	+6°
2 days.....	160	51	160	51	51	51	51	51
4 days.....	5100	90	2800	90	67	38	67	38
8 days.....	9000	900	3800	2100	210	38	380	—

Control: AST after 8 days at room temperature = 51 units.

Table 2.

Serum sample 2743. AST before contamination = 67 units.

Titration after	Fl. 1		Fl. 2		P. 1			P. 2		
	+20°	+6°	+20°	+6°	+37°	+20°	+6°	+37°	+20°	+6°
2 days.....	160	67	210	90	—	90	67	—	90	90
4 days.....	2100	120	900	160	510	120	67	1600	280	90
8 days.....	5100	120	3800	160	38000	3800	67	67000	—	67

Control: AST after 8 days at room temperature = 67 units.

Table 3.

Serum sample 4138. AST before contamination = 51 units.

Titration after	Fl. 2		
	+37°	+20°	+6°
3 days.....	120	900	380
7 days.....	510	5100	900
Control: AST after 7 days at room temperature = 67 units.			

As will be seen from the tables, the *fluorescens* strains caused the greatest increase in the antistreptolysin titre at room temperature, but a noticeable effect was also produced at 37° C. and at 6° C. With the *pyocyaneus* strains, on the other hand, the greatest change in the titre occurred at 37° C.; there was a slighter change at 20° C. and none at all at 6° C., after an observation time of one week. The differences can probably be explained wholly by the different temperature optima of these bacterial strains.

Similar experiments were carried out using other bacterial strains. Strains of *Bacillus subtilis* were found to possess a similar power — although not always — to cause a rise in the antistreptolysin titre. Table 4 shows the results of experiments with two *subtilis* strains, called S. 1 and S. 2.

Table 4.

Serum sample 4137. AST before contamination = 51 units.

Titration after	S. 1			S. 2		
	+37°	+20°	+6°	+37°	+20°	+6°
3 days.....	2800	670	38	670	280	51
7 days.....	5100	3800	51	6700	3800	38
Control: AST after 7 days at room temperature = 67 units.						

The rise in the antistreptolysin titre as a result of the addition of *subtilis* strains was thus greatest at a temperature of 37° C., a little less at 20° C., and at 6° C. there was no change at all, after one week's observation.

Other bacterial strains (*staphylococci*, *streptococci*, *coli*, *proteus*) gave no measurable rise in the titre at 20° C. and 6° C. A slight increase was obtained in some instances, on the other hand, after the serum had been incubated at 37° C.

Both the *pseudomonas* and the *subtilis* strains made the serum

anti-complementary in the Wassermann reaction. The Widal reaction, on the other hand, was not influenced by the contamination.

Discussion.

The fact that bacterial contamination of serum samples can cause a rise in the antistreptolysin titre is not only interesting from the theoretical point of view, but it is also of practical importance. No corresponding phenomenon seems to be known with regard to other serological reactions. As we know, serum is made anti-complementary in the Wassermann reaction, as a result of contamination with certain types of bacteria, particularly with those belonging to the *subtilis* group (*Bacillus cereus*). The change in the reaction in this case does not lead to incorrect diagnosis, however. According to *Sievers*, *B. cereus* exercises its influence on the Wassermann sample by attacking the third component of the complement. My experiments proved that *Ps. pyocyanea* and *Ps. fluorescens* were also capable of making serum anti-complementary.

The sensitivity of the antistreptolysin reaction to bacterial contamination is in all probability linked up with the special properties of streptolysin, in particular with its sensitivity to oxidation. The pigment formed by *Ps. pyocyanea*, pyocyanin, is known to be a respiratory ferment. Some investigators, however, have made observations indicating that *pyocyaneus* strains which do not form pyocyanin can also have a similar fermenting effect (*Reed and Boyd*). Thus, if this fermenting property is not necessarily associated with pyocyanin, it is conceivable that it may instead be common to both *Ps. pyocyanea* and *Ps. fluorescens*, the last-named type of bacteria also being, as we know, a pigment-producer. There seems justification for assuming, therefore, that these bacteria, when growing in serum, form an enzyme, which, on the addition of streptolysin, oxidizes this and thus inactivates it.

The question of whether the similar effect of *B. subtilis*, and possibly also of other bacteria, can be explained in the same way must for the present be left unanswered.

In whatever manner the effect of the contamination on the antistreptolysin reaction is explained in theory, its practical significance is obvious. As regards *Ps. fluorescens*, as already mentioned, the effect has been demonstrated in samples of blood which have been sent to us. Considering that this type of bacteria commonly occurs in air, and that it will also grow at low temperatures, one must take into account that it may constitute a source of error in antistreptolysin determinations. Although the biological effect of *Ps. pyocyanea* in this respect is very similar, its practical importance is probably slight. The significance of contamination with *B. subtilis* is not yet clear,

but in all probability it affects the samples to a lesser extent than *Ps. fluorescens*.

In the investigations which have been made hitherto in connection with antistreptolysin it has presumably been possible, as a rule, to carry out the titrations soon after the samples have been drawn, or, when this was not the case, to keep the samples of serum in a frozen condition in the interim. In both of these cases, if contamination has occurred, the consequences are in all probability eliminated. But it is obvious that as the antistreptolysin reaction becomes more widely known and practised, samples will be sent in for examination from long distances away. The samples will be exposed to unfavourable temperatures in transit, especially in summer-time, and if they become contaminated with bacteria it is likely that quite considerable errors may be made in the determinations.

If this source of error is to be avoided, it is essential that sterile conditions should prevail during the taking of the samples. If a long interval is to elapse between when the samples are taken and when the titrations are carried out, the serum should be kept frozen. Finally, it should be stressed that the repeated taking of samples is an indispensable control. Particularly in the case of antistreptolysin titration it is risky to draw conclusions from single samples. As with other serological reactions, a sample giving a normal value may have been taken at a phase before antibodies had had time to form, and thus need not be an indication that no infection is in progress. An isolated sample showing a high antistreptolysin titre may be due, under certain circumstances, as has been demonstrated in the present paper, to bacterial contamination, and thus can not be made the basis for an etiologic diagnosis. On the other hand, if a series of samples are taken at not too long intervals apart, it is possible to judge more accurately whether the results are to be trusted and whether they will justify a statement as to the etiology of the case.

Summary.

A simplified method of titration for antistreptolysin determinations is described, according to which the sera are diluted in a series with a logarithmic increase of 0.125.

The author demonstrates how contamination of blood samples with *Pseudomonas fluorescens*, *Pseudomonas pyocyanea*, and *Bacillus subtilis* can cause an excessive rise in the antistreptolysin titre.

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MECHANICAL TRAUMATISM AND DEVELOPMENT OF TUMORS IN INBRED MOUSE STRAINS.

By *J. Engelbreth-Holm.*

(Received for publication September 1st, 1944).

The question whether a mechanical traumatic injury may induce the development of a malignant tumor may still be said to be unsettled conclusively even though recent investigations with increasing certainty indicate that a single traumatic injury is not able to elicit any growth of tumors.

This applies also to the forms of tumors which not long ago were taken to develop not infrequently on the basis of traumatic factors, namely: tumors of the testis, sarcoma of bones, and carcinoma of the breast.

Recently, in this country, Poulsen (1942) has dealt with this question on the basis of patient materials from the Directorate of Accident Insurance in Copenhagen and from the Radium Station in Copenhagen. Poulsen found no definite evidence of the etiological significance of traumatic injury in cases of testicular tumors and sarcoma of bones, even though such a connection naturally could not be excluded in a few of the cases — just as it is impracticable in this way to prove the negative answer to the question: a lack of such connection. Poulsen concludes that in most of the cases the tumor now has to be taken as having existed prior to the traumatic injury which attracted the attention of the patient to the disease.

In the same work Poulsen further reviewed the animal experiments reported in the literature and arrived at the same result: that a traumatic injury cannot be assumed to constitute any etiological factor of importance in the development of tumors. He emphasizes, however, that such experiments have been too few and usually carried out on too few animals to be of any conclusive significance.

Furthermore, the serviceability of the animals employed for experiments of this kind may be disputable. As far as that goes, the same applies to innumerable tumor experiments of an earlier date. As a matter of fact, which has not been realized until recently, in previous

experiments on the induction of malignant tumors through exogenous influence, all too slight attention has been paid to the character and constitution of the animals employed. The classical experiments with production of skin cancer in mice by painting them with tar — an experiment which actually turns out successfully on practically all kinds of mice, even though with varying readiness — gave rise to the erroneous view that »cancerigenic« treatment might induce tumor growth in practically all tissues in all animals. It was soon realized, however, that the matter is not so simple as that. But it is not generally known that — as is evident, among other things, from experiments reported during the last years from the University Institute of Pathologic Anatomy — the matter really stands that cancerigenic treatment presumably is able to induce tumor growth only in a given tissue in a given animal species if the animal concerned is able through hereditary disposition to develop a tumor in the tissue concerned.

For instance, it is practicable to produce cutaneous carcinoma and fibrosarcoma in nearly all mice, it is true, but if we try to induce tumor growth in other tissues — lungs, kidneys, liver, testes, etc. — and for these experiments we employ mice of strains which without any treatment are »tumor-free« in these organs, the experiments are most likely to turn out negative. This is illustrated very well by Esmarch's*) work, in which he tried to induce tumor growth by injection of methyleholantrene into various organs in mice of Bagg's strain. Bagg's strain is an inbred mouse strain which is characterized by being »tumor-free«. In his experiments, indeed, Esmarch obtained merely the result that only such tumors developed which are seen to occur spontaneously now and then in these mice. Experiments of this kind may be said to have been planned inexpediently insofar as they cannot be expected to yield any other tumors than squamous epithelioma of the skin and sarcoma arising from the connective tissue. Indeed, the result of these experiments was that the application of methyleholantrene to the mammary glands, musculature, kidney, liver and lung, merely induced sarcoma from the connective tissue and carcinoma of the skin.

Also in experiments on production of tumors by traumatic agency these aspects of the animals should be taken into consideration. This has not been done in the experiments reported hitherto, where traumatic injury simply was applied to one tissue or other in practically accidental experimental animals.

Only Slye (see the survey given by Poulsen) worked with mice whose disposition to tumor growth was known, but his experiments are difficult to interpret, and adequate controls appear to be lacking.

*) Esmarch, O.: Studier over Metylcholantren og dets kræftfremkaldende virkning paa Mus. Munksgaard, Copenhagen, 1940.

On working with inbred mouse strains whose spontaneous tendency to tumor development is thoroughly known, we have been able here in the University Institute of Pathologic Anatomy to show that exogenous carcinogenic agents are able in a high degree to promote («accelerate») the development of the tumors characteristic of the strain, whereas other tumors fail to appear. Thus, in a leukemic mouse strain, called Aka, the development of leukemia or lymphosarcoma may be accelerated by painting the skin with carcinogenic hydrocarbon,*)**) while in another strain, named Street, with a tendency to development of mammary carcinoma, a similar treatment may promote the development of this form of cancer.***)

So the idea suggests itself to investigate whether severe traumatic injury — contusion of the tissue or lymph gland in mice of the Aka strain, and the same treatment of the mammary gland in females of the Street strain — will be able to promote the development of tumors in these animals, especially whether such treatment will bring about a tumor growth — respectively lymphosarcoma and mammary carcinoma —, at the site of the traumatic injury.

Experiments with Contusion of Lymph Nodes in Mice of a Leukemic Strain.

In mice of the Aka strain, in which leukemia develops spontaneously in 57 % of the animals, an inguinal lymph node was crushed by pressure between two fingers. Such contusion of the lymph nodes can be felt distinctly, the round firm lymph node bursting between the fingers. The contusion was performed 5—6 months before the spontaneous development of leukemia reaches its maximum.

To the relevance of this experiment, perhaps, the objection may be raised that it cannot be taken for granted that autoctonous development of leukemic foci in the lymph nodes arises through the development of the leukemia, and that changes in the lymph nodes are due to immigration — »metastasis« — from some other place, so that a traumatic injury to a peripheral lymph node possibly does not involve at all the tissue which primarily is changed by the development of the leukemia.

Traumatic injury to a lymph node in the left groin was performed on a total of 44 animals. Among these animals, leukemia of the usual type developed in 30 at the usual time. Thus the incidence of

*) J. Engelbreth-Holm: »Beschleunigung der Lymphosarkomatoseentwicklung bei Mäusen«. Folio Haematol. 63: 1940.

**) J. Engelbreth-Holm & H. Lefevre: »Om accelerering af udviklingen af spontane svulster hos mus«. Ugeskrift f. Læger 103: 1941.

***) J. Engelbreth-Holm & O. Poulsen: »Accelereret udvikling af spontan leukemi og mammacarcinom hos mus efter sondefodring med carcinogen kulbrinte«. Ugeskrift f. Læger 43: 1943.

leukemia among these animals was 68 %. But among the 11 untreated controls, which were sibs of the treated animals, the incidence of leukemia was equally high percentally (7 animals). Evidently, then, the litters employed in this experiments have had a somewhat greater tendency to leukemia than the average for the total Aka strain. This finding, that the incidence of leukemia may vary somewhat from generation to generation is well known, and it necessitates the employment of sibs for controls.

In no instance was the development of lymphosarcoma at the site of the traumatic injury observed among the treated animals. Nor did the contused lymph node turn out to become leukemic prior to the other lymph nodes; nor did it become larger than the others. The treatment has had no influence whatever on the development of leukemia in these animals.

Experiments with Contusion of a Mammary Gland on Mice of a Mammary Carcinoma Strain.

On female mice of the Street strain, among which spontaneous mammary carcinoma is seen in 24 %, a mammary gland in the left groin was crushed by means of Péan forceps. In this experiment no attempt was made at contusion of the gland with the fingers because it requires a much greater force to crush the mammary tissue than a lymph node.

Contusion of the breast was performed on 49 females 8—9 months prior to the maximal age for the spontaneous development of these tumors. This treatment was tolerated without any reaction apart from rather small hematomas which were absorbed rapidly.

Among these 49 mice, mammary carcinoma developed at the usual time in 10 animals (about 20 %) that is, not more frequently than observed for the strain in general, and not more frequently than encountered among the untreated control sibs, among which mammary carcinoma was observed in 6 out of 24 mice (25 %).

In the 10 treated animals with tumor growth altogether 13 mammary carcinomas were observed. Of these 13 cancers 4 were localized to the left inguinal region, that is, possibly arising from the contused gland, whereas two-thirds of the tumors developed more proximally on the left or right side. As the size of the tumors — most often about 2 cm. in diameter — does not allow of a more accurate localization than the »upper« or »lower« half of the milk line, by accidental distribution about one-fourth of the tumors develops distally on the left side.

In agreement with this, among the controls 8 tumors developed in 6 animals, and of these 8 tumors exactly one-fourth (2) developed in the left inguinal region. In the treated animals 4 out of 13 tumors were located in the left inguinal region — that is, this localization

was somewhat higher among the treated animals. It will hardly be justifiable, however, from these findings to conclude that the traumatic injury has resulted in an increased tendency to tumor development. The figures are too small to allow of any such conclusion. The decisive point must be that among the 49 treated mice the appearance of mammary carcinoma was no more frequent than observed among other mice of this strain, especially the controls.

So the outcome of these experiments must be that the traumatic injury has not resulted in any unquestionable increased incidence of mammary carcinoma.

Experiments with Contusion of Testis.

Even though the occurrence of spontaneous testicular tumors has not been observed in the mouse strains here employed, a number of experiments were carried out in which the testis was contused by pressure between two fingers. If this treatment had resulted in tumor growth the outcome would have constituted conclusive evidence. After what has been emphasized in the introduction concerning the chance of producing tumor growth through exogenous agents, it is of minor interest that contusion of the testis in 46 animals of the Aka and Street strains did not induce tumor growth in any instance. On microscopic examination of the contused testis at the death of the animals, as a rule several months after the traumatic injury, only remnants of necrosis were seen together with development of connective tissue.

But, as mentioned already, presumably it is not to be expected at all that testicular tumors may develop in these strains, and hence the negative outcome of the experiments is of slight significance or none at all.

As a *conclusion* of these findings it may be said that considerable traumatic injury (contusion) to lymph nodes in 44 mice with a hereditary tendency to leukemia and to mammary tissue in 49 mice of a mammary carcinoma strain has no tumor-promoting effect.

Nor has contusion of the testis in mice without spontaneous development of testicular tumor brought about any tumor growth in 46 animals.

Concerning the literature see:

O. Poulsen: Traume og malign Svulst. Bibl. f. Læger. 1942. 377.

SOME OBSERVATIONS ON THE FIBRINOLYTIC FACTOR IN HUMAN SERUM

By Carl G. Holmberg.

(Received for publication September 27th 1944.)

Milstone (1) showed in 1941 that the streptococcal fibrinolysin for its action necessitates the presence of a factor occurring in the human serum and precipitated with the euglobulin.

Aiming to localize this factor more closely, we have fractionated the euglobulins from human serum according to Green (2). By this technique two fractions are obtained primarily, one precipitated at pH 6.5 and the other at pH 5.0. Green called the first fraction $P_{II} + P_{III}$ and the second P_I . Both these fractions contain about the same amount of lytic factor, in spite of the absolute amount of P_I only equalling about $1/4$ of $P_{II} + P_{III}$. It is not possible by repeated precipitations to obtain any certain displacement of activity between these two fractions.

By dissolving the $P_{II} + P_{III}$ -fraction in diluted acid and then neutralizing it to pH 5.0 a precipitate of P_{III} is obtained, while P_{II} is precipitated if the neutralization is continued to pH 6.2. Of these two fractions P_{II} is practically non-active, while P_{III} contains most of the activity.

Svensson (3) has investigated Green's fractions cataphoretically and found that the correlation is roughly the following: $P_I = \alpha$ -globulin, $P_{II} = \gamma$ -globulin and $P_{III} = \beta$ -globulin. The lytic factor should thus mainly be found within the α - and β -globulins.

As the α - and β -globulins are richer in lipoids than the γ -globulin the lytic factor might be assumed to be a lipid. To investigate if such might be the case, a solution of P_{III} was precipitated with the 20fold amount of acetone, after which the precipitate was extracted with ether. The protein treated in this way showed a practically unchanged lytic activity. If a lipid component is a part of the lytic factor, it must consequently be relatively closely bound to the globulin.

In all these investigations the test system employed has been the combination horse fibrinogen + horse thrombin + concentrated streptococcal fibrinolysin. This combination shows some spontaneous fibrinolysis, but is still quite suitable as a test system.

The system here described has also been used to determine the amount of lytic factor in various sera after precipitation according to Milstone. We found that this amount kept very constant. A series of 7 normal sera showing no significant variation in titer. It is rather interesting that the serum from a 2-months-old child showed exactly the same titer as the serum from adults. *Lippard* and *Wheeler* (4) and *Lippard* and *Johnson* (5) have as a matter of fact shown that plasma from children up to the age of 3 months is strongly resistant to streptococcal fibrinolysin. This resistance would thus seemingly not depend on the absence of lytic factor.

One patient with erythema nodosum on a definitely established rheumatic basis showed a quite normal titer, too. We finally examined a patient who had been given 3-3-methylen-bis-4-oxycoumarin (prothrombin index 40) for some time. The titer was found to be normal also in this case.

As an example of the technique for determining the lytic factor an experiment, aiming at illustrating the amount of lytic factor in child's serum as compared with serum from adults, is described below.

2 series of 5 micro-test tubes are prepared in the following way. Each tube gets: Lysin dil. 1/20 0,25 ml. + buffer pH 7,5 m/15 0,2 m. l. + horse fibrinogen 0,1 m. l. + 2 drops of horse thrombin. To the first tube in the first series is added 1 drop of euglobulin, precipitated from child's serum according to Milstone and dissolved in phosphate buffer to the original volume of the serum. To the second tube is added 1 drop of the same euglobulin, diluted to 1/5, to the third tube dil. 1/10 and to the fourth dil. 1/40. The fifth tube receives a drop of water. The second series is treated in the same way with euglobulin from adult normal serum. The tubes are put in a water bath at 37° C. and the time necessary for the clot to dissolve is read off. The results are given in the tables below.

Child's serum		Normal serum from adult	
euglobulin undiluted	dissolv. time 5 min.	euglobulin undiluted	dissolv. time 7 min.
1/5	11 »	1/5	13 »
1/10	21 »	1/10	25 »
1/40	29 »	1/40	29 »
1/∞	60 »	1/∞	60 »

Summary.

In precipitation according to Green the greater part of the lytic factor goes to the euglobulin fractions P_I and P_{III} .

The amount of lytic factor in human serum has been determined under varying circumstances and found to be very constant.

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HEREDITARY TUMOR-LIKE TAKES IN TRANSPLANTATION OF LEUKOSIS IN MICE*)

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(Received for publication October 3rd 1944).

In the last couple of decades, experimental genetic studies have played a not inconsiderable role in research in malignant tumors, including various forms of leukosis. Gradually and increasingly investigators have realized the significance of the genotype to the development of various forms of tumors in connection with other known or unknown factors. Primarily, it was spontaneous tumors that interested the investigators, but as transplantation of tumor tissue and leukotic tissue to a large extent finds employment in various experiments, geneticists have become interested also in the inheritance of susceptibility to the transplantation of malignant tissue.

Briefly, the outcome of these investigations has been that the susceptibility to transplanted tumor tissue is dependent on the presence of one or more dominant genes, characteristic of the tumor line in proportion to the strain of animals employed.**)

Most investigations on the genetic aspects of leukosis have been carried out on mice as it has been established gradually that certain strains of mice present a very high susceptibility to spontaneous leukosis, especially on inbreeding. Particularly *MacDowell & Richter et al.* have contributed to elucidate the problems mentioned through very extensive experiments on a mouse strain C 58, with a high percentage of leukosis, most often lymphogenous, with only a small percentage of myelogenic leukosis (*Mac Dowell & Richter*, 13). In their work an account is given of the heredity of the spontaneous leukosis, the strain being crossed with another strain StoLi, which is characterized by the very high frequency of mammary cancer, while the incidence of leukosis is very scarce. Thus, in the first filial generation the incidence of leukosis is found to be halved, though in this way that the offspring after the crossing of StoLi ♂—C 58 ♀ get the disease

*) The studies here presented were carried out with the aid of a grant from *Anders Hasselbalchs Leukæmifond*.

**) In the present work the leukotic tissues which are transplanted and sometimes carried further through several passages will be designated as a transplantation line.

in 61.9 % while the converse crossing gives offspring that get the leukosis in 42.5 %.

The same phenomenon is seen on back crossing to the original strains from the first filial generation. At a glance the outcome looks like a sex-linked inheritance, but as the difference in the frequency is distributed equally on males and females in the offspring, it cannot be a question of a sex-linked, chromosomal inheritance. More likely, a cytoplasmic factor plays a contributory role, associated especially with the ovum.

When the animals in the original strain C 58, which has been inbred for more than twenty generations, and thus may be considered as almost completely homozygotic, show an incidence of leukosis amounting to 90 %, this has to be attributed to a failure in the capacity for manifestation, as offspring of the 10 % negative animals behaved in this respect exactly as the positive.

In the first filial generation, as mentioned, all the individuals are genotypically identical and yet leukosis makes its appearance only in about half of them. Here the manifestation capacity is even lower than in the original strain. Thus it is evident that the relation between the hereditary and the unknown extraneous factors still remains obscure.

MacDowell & Richter's experiments on the susceptibility to *transplanted leukosis* (8, 9, 10, 11, 12, 14, 16, 17, 18) revealed interesting facts concerning this question. In 1929, for instance, MacDowell & Richter (16) reported their first experiments with transplantation of a spontaneous case from strain C 58 on mice of the same strain.

In subsequent works (17, 8, 9 and 10) these authors reported the results of a more thorough genetic analysis of these problems. Strain StoLi proved resistant to transplantation of leukotic tissue from strain C 58, whereas C 58 showed 100 % susceptibility to transplantation of the same material. The first filial generation of the crossing between C 58 and StoLi showed 100 % susceptibility, whereas the second filial generation gave takes in 79.5 % of animals. Back crossing from the first filial generation $F_1 \times C\ 58$ gave 100 % susceptibility whereas $StoLi \times F_1$ showed a susceptibility of 48.2 %. These figures do not differ with statistical certainty from the figures that would be obtained theoretically if the susceptibility is assumed to depend on one dominant gene.

This, however, applies only to a certain transplantation line (line I). On comparison between different lines the authors (18) found some difference in the percentage of takes. According to these investigations, then, one cannot speak of any definite inheritance in general for the susceptibility, only for the individual cases.

The investigations cited here are exclusively of quantitative nature. The authors mention that a few spontaneous cases pathologic-anatomically were to be classified between generalized leukosis and lymphosarcomatosis or lymphosarcoma-like cases (17). In their material no distinction is made between these forms, however, as the authors have preferred to employ the term »leukemia« as common designation.

The picture developing after transplantation is described by the authors as generalized leukosis. In one of their works (Richter & MacDowell, 18) the authors were able by comparison between 4 transplantation lines to establish that each line develops a fairly constant picture that is characteristic of the line, whereas between the lines there are rather considerable variations with regard to the white blood count, extension of the infiltrations in the liver, kidneys and spleen, exudates in the pleural and peritoneal cavities, besides the length of survival after the transplantation. From this it is evident, then, that the variations are primarily dependent on differences in the transplanted leukosis cells.

Schweitzer and *Furth* (19) were able to confirm the results of *Mac Dowell* and *Richter* in experiments with two strains of mice, Ak and Rf, with respectively high and low incidence of spontaneous lymphogenous leukemia. The presence of one or two dominant genes in the host was necessary for the growth of the transplant.

No variation occurred in the pathologic-anatomical picture of the hybrid mice.

Also other authors have described variations in the pathologic-anatomical picture on transplantation of leukosis, as some forms completely remind of spontaneous generalized leukosis, while other forms remind of isolated tumors (lymphosarcoma), and then there are various intermediate forms.

The various authors take this fact to prove that the corresponding forms in spontaneous cases are very near-related. Even though this presumably is correct, the possibility cannot be excluded that here we may be dealing with a false conclusion of analogy, as we cannot a priori know whether the spontaneous and the transplanted forms in their origin depend on the same factors.

Thus in 1924 *Snijders* (21, cited after *Tio Tjwan Gio*) reported the outcome of a number of transplantations from a spontaneous instance of lymphogenous leukosis in an East Indian guinea-pig performed on animals of the same strain. In 219 cases (56 %) the animals showed an entirely leukotic picture with increase in the cell count. In 48 cases (12 %) the same pictures was seen but together with some infiltration at the site of transplantation. In 62 cases (16 %) the changes reminded of aleukemic lymphadenosis. 62 animals (16 %) showed pictures similar to leukosarcomatosis (*Sternberg*). In 14 cases the findings were described as aleukemic leukosis with local tumor growth; finally, 14 animals (4 %) showed only an entirely local growth of the transplant.

In 1927 *Tio Tjwan Gio* (22) reported similar findings when he was able to transmit the same leukosis line as *Snijders* had worked with to guinea-pigs of Dutch origin.

In both instances the guinea-pigs belonged to definite strains, it is true, but no information is given about the inbreeding. Nor was any genetic analysis proper carried out, so that the authors had to be content to ascertain the facts mentioned.

In 1929 *Korteweg* (6) reported a number of transplantations from a spontaneous case of lymphogenous leukosis in an accidental laboratory mouse which 11 months before in another experiment had been treated intratracheally with tar. The leukosis was found to take in 154 of 628 animals. A characteristic feature of the take was the development of a local tumor at the site of transplantation, regardless whether the transplantation was performed subcutaneously, intraperitoneally, in the kidney region or in the splenic region, and that this local tumor was accompanied by more or less extensive leukotic changes in the various organs. The author mentions explicitly that the animals employed were not well defined genetically.

Korteweg designates takes that are characterized by local tumor-like growth without extensive generalization or blood changes as lymphosarcoma, while he classifies the other generalized cases (tumor, generalized changes and blood changes as leukosarcomatosis)

Among the best experimental works on leukosis mention is further to be made of a number of papers published by *Furth and collaborators* (2, 3, 20). In these experiments the material consists of spontaneous cases of lymphogenous leukosis developed in 3 different strains — A, R and S. None of the strains have any particularly great frequency of leukosis, and at the commencement of the experiment none of them were inbred in such a degree

that they may be considered homozygotic. Indeed the authors describe how several branches of the A strain show different properties.

In one paper (*Furth, Seibold & Rathbone*, 3) a description is given of 5 spontaneous cases of lymphatic leukosis and the transplanted cases developed herefrom. The transplantations were performed subcutaneously or intravenously. A feature common to all the lines was that more takes were obtained with the intravenous transplantation than with the subcutaneous. Furthermore, in all the lines the intravenous transplantation resulted in generalized leukosis (leukemic or aleukemic), whereas the subcutaneous transplantation resulted in a local tumor which in some cases was accompanied by general changes in lymph glands and parenchymatous organs, sometimes also with blood changes. From this it is evident that the picture largely depends on the technique of transplantation or, as the authors put it, on the chance for the leukotic cells to enter the blood stream.

According to these investigations too, the outcome is somewhat dependent on the transplantation line employed (cf. *Richter & Mac Dowell*), as there are characteristic differences between the lines. Thus, three of the lines (Rg 10, Ak 30 and Ar 21) show often on intravenous transplantation not only the leukosis-like features but also tumors in the muscles or in the kidneys. In addition, a number of less important differences are described, among others, in the white blood count, which appear to be somewhat dependent on the count in the original spontaneous case, a high original count giving high figures in the transplants.

As to the susceptibility to the transplantation, the strain of animals in which the original case was found proved also to be the most susceptible: 3 lines give takes only in animals of the respective strains, while 2 others give takes in all the 3 strains mentioned. The conclusion of this will be that the take depends on the technique of transplantation employed and on the character of the leukotic cells. The experiments were carried out on a genetically not well-defined material, so that the hereditary factors cannot be checked up.

In 1935 *Furth* (4) published a paper on a case of leukosis of unquestionable myeloid nature. In transplantation experiments performed in various ways (intravenous, intraperitoneal and subcutaneous injection) on mice irradiated with X-rays, takes were obtained in a great part of the animals. Intravenous transplantation gave most often a picture of generalized myelogenous leukosis. The two others methods of transplantation gave most often a local tumor formation. In the latter cases, the period of survival was considerably longer than in the animals with a generalized form.

But, in a few cases, the intravenous transplantation resulted also in local tumor formation instead of generalized leukosis. Under the same conditions, tumors were found more frequently in non-irradiated mice than in the irradiated. As the X-ray treatment lowers the resistance of the animals, the author arrives at the conclusion that the tumor-like picture obtained signifies a greater resistance and that the outcome of transplantation, therefore, is dependent on the resistance of the animal.

In experiments with intravenous transplantation on irradiated mice with decreasing doses of transplant, the author observed that when only a few cells are injected the result is the development of solitary or multiple tumors.

Danish investigators have published a number of works on the transplantation of leukosis in mice but not brought any essential new findings concerning the morphological or genetic aspects of the problem that have not been reported by American authors.

Thus, in 1930 *Krebs, Rask-Nielsen & Wagner* (7) reported their studies on a number of spontaneous cases of leukosis in non-inbred laboratory mice. The incidence of the lesion was higher in the animals which had been

given X-ray treatment. A thorough description is given of the cases and at that time the authors took the lesion to be lymphogenous leukosis.

With these spontaneous cases of leukosis the authors tried to produce transplantation lines, and in a few lines they succeeded in this but only on mice that had been irradiated beforehand. The outcome of these experiments was a number of cases which according to the authors, may be compared to the following four groups of lesions known from the spontaneous cases and from human pathology:

- I. Leukemic lymphadenosis
- II. Aleukemic lymphadenosis.
- III. Lymphosarcomatosis (Kundrat).
- IV. Leukosarcomatosis (Sternberg).

The authors suggest the term «lymphomatosis infiltrans (leukæmica et aleukæmica)» as a common diagnosis for the various pictures observed — both for spontaneous cases and for cases produced by transplantation.

Subsequent investigators (*Kaalund-Jørgensen* and *H. Chr. Rask-Nielsen* & *R. Rask-Nielsen*) have worked with the same line but, in contrast to the previous authors, they look on the disease as myelogenous leukosis, as they found a few peroxydase-positive, immature cells in the peripheral blood of the transplanted animals, and furthermore, in the final stage of the disease after the transplantation there is a pronounced neutrophil leukocytosis prior to the appearance of the leukotic cells in the blood. The leukotic cells proper are described as myeloblasts. One has to be cautious, however, in drawing any conclusion from the finding of immature myeloid cells in transplanted leukosis, as the possibility of a reaction on the part of the bone marrow cannot be excluded. The neutrophil leukocytosis does not signify a formation of neutrophil leukocytes from the leukotic cells — but just an irritation of the bone marrow.

Thus, in 1936 *H. C. Rask-Nielsen* & *R. Rask-Nielsen* reported a number of transplantations carried out with this line and found in some of the cases a picture resembling generalized leukosis with changes in the blood and bone marrow. Less frequently there developed merely a local tumor at the site of the transplantation without generalization or changes in the blood or bone marrow.

In 1936 *Kaalund-Jørgensen* (5) has described a similar transplantation experiment with the same line. Here he succeeded for the first time in transmitting this leukosis by subcutaneous or intravenous transplantation on mice which had not been treated with X-rays. When the transplantation of this line has been rather difficult it is due entirely to the circumstance that the experiments were carried out on accidental mice bought from mouse farms. On transplantation the author obtained the following three pictures.

I. A local tumor at the site of injection, accompanied by infiltration of the surroundings, sometimes with swelling of the regional lymph glands, but without generalized leukosis or changes in the blood and bone marrow.

II. Local tumor at the site of injection, accompanied by universal glandular swelling and changes in the organs, but no changes in the blood or bone marrow.

III. The same picture as in II plus changes in the blood and bone marrow.

The author arrives at the conclusion that the different pictures signified the same lesion, and that the outcome depends on different factors: 1) the growth of the cells, 2) the susceptibility of the animals, and 3) the method of transplantation.

This conclusion might apply to the result of the investigations in this

field cited here. In which way the genotype of the animal influences the result is not indicated. In the work to be presented here this is just the question that has been investigated, as efforts have been made to keep the other factors constant or under control. Besides, all the experiments have been carried out exclusively with mice of known breed.

Finally we must refer to a work by *MacDowell and Richter and collaborators* dealing with the influence of the resistance of the host on the pathologic-anatomical picture. It is known that complete resistance against a given line of transplanted leukemia may be induced by treating the susceptible mice with small doses of leukemic cells of the line or with normal embryonic tissue of a certain genetic structure.

In 1936 *MacDowell, Potter et al.* (12) reported an interesting phenomenon concerning the variation of the pathologic-anatomical picture of transplanted leukemia. In mice treated with embryonic extract developed, after inoculation with leukemic cells, multiple or single lymphosarcomatous tumors in various parts of the body, sometimes accompanied by real leukemic lesions in other organs.

Histologically they show a large proportion of connective tissue contributed by the host. The malignant cells were markedly different from those of the line inoculated.

These tumors were transplanted further on normal untreated mice, and in most cases, the authors observed development of leukemic lesions characteristic for the line, but in one case the tumor continued to reappear in all mice in 5 successive transfers.

It is clear that the resistance of the mice treated with embryonic tissue has modified the character of the pathologic-anatomical picture temporarily or in certain cases permanently.

Experimental.

In order to investigate the inheritance of the susceptibility to transplanted leukosis, here in the University Institute of Human Genetics a crossing has been carried out between the Aka strain and the B strain.

The Aka strain is a purely inbred albino strain characterized by very frequent occurrence of spontaneous leukosis. In most of the cases the nature of the leukosis cannot be determined more definitely as the pathological cells are of such an immature character that they are rather to be designated as stem-cells. In a few cases, however, it is possible to recognize a differentiation of the cells in myeloid or lymphatic direction.

The macroscopic picture is somewhat varying. In most cases there is a rather pronounced universal swelling of the lymph glands and enlargement of the spleen, liver and thymus. Microscopic examination shows infiltration of the organs concerned with leukotic cells. Not infrequently the most conspicuous change is an enormous enlargement of the thymus, which occupies a large part of the thorax, crowding the heart and lungs. As a rule, however, a slight enlargement of the peripheral lymph nodes can be made out at the same

time, and microscopy shows some infiltration of the various organs (kidneys, liver and spleen) even though they present no distinct macroscopic changes. In a few cases, the mice present merely a slight peripheral glandular swelling, while the liver and spleen are greatly enlarged.

The B strain is a not completely inbred strain characterized by the occurrence of recessive dwarfism. The strain has been bred here in the institute for several years without any admixture of other strains. Sibling breeding is of frequent occurrence, so that it is safe to reckon with some degree of homozygosis. In the crossing of the two strains it was aimed, for one thing, to investigate how the leukosis behaves in an organism with hypophyseal insufficiency as manifest in the dwarf mice.

The crossing was extended to the 2' filial generation. From this generation a number of strains were formed by accidental choice of males and females for the breeding; and these strains have then been carried farther by pure sibling inbreeding.

In the transplantation experiments individuals from both main strains were transplanted as well as individuals from the 1' and 2' filial generations. Further, from the strains from the 2' filial generation litters were picked out from the various generations and tested for the susceptibility to the transplantation. The material used for the transplantation was leukotic tissue from 14 spontaneous cases in the Aka strain (see Table 1).

On macroscopic examination all the spontaneous cases presented about the same features. There was some variation in the degree of the enlargement of the axillary, cervical and inguinal lymph nodes. The mesenteric lymph nodes were always greatly enlarged, sometimes even to a monstrous size, but there has never been infiltrative growth in the surrounding tissues. Also the retroperitoneal lymph nodes along the large vessels have been enlarged in varying degree. As a rule there is some enlargement of the liver, and the spleen is constantly enlarged. Often the liver and spleen are somewhat lighter in color than normally and sometimes they present a surface speckled with leukotic changes. In several cases the thymus is found to be very large, and there is also enlargement of the mediastinal lymph nodes. In other cases there is merely a moderate enlargement of the thymus. As a rule, the leukotic lymph nodes and thymus are whitish in color, and small hemorrhages into the tissue are infrequent. In no instance was necrosis observed, not even in the largest lymph nodes.

On microscopic examination the organs mentioned show infiltration with leukotic cells in varying degrees. The lymph nodes and thymus show complete effacement of the normal structures. The tissue consists exclusively in close-packed leukotic cells with only a scanty stroma and few blood vessels. The liver presents most often a very pronounced perivascular infiltration, but at the same time

there is also a good deal of diffuse infiltration, so that the trabeculae of liver cells are forced apart by small accumulations of leukotic cells. The spleen shows a very dense infiltration; none of the normal follicles can be made out. Also the kidney shows perivascular infiltration and some infiltration between glomeruli and tubules; in addition, an accumulation of leukotic cells in the glomeruli themselves is a frequent finding.

The leukotic cells are fairly large, round or polygonal, the form being somewhat dependent on the location of the cells. The protoplasm is rather scanty, the nucleus large with a loose chromatin net. The occurrence of distinct large nucleoli is very characteristic; the nucleus is either round or slightly notched, as a rule located a little excentrically. In specimens stained *ad modum* Giemsa the protoplasm appears very strongly basophil, sometimes with a perinuclear clearing. Granules are never found in the cells. In imprint-specimens, fixed with May-Grünwald's solution and stained with Giemsa's solution, beautiful pictures may be obtained from the changed organs, showing distinctly the leukotic cells mentioned.

In addition, white blood counts have been made with differential count on blood smears stained after Giemsa.

Apart from two of the spontaneous cases, all the cases have been aleukemic, as the cell count has not exceeded 25,000. Still, it has always been practicable to demonstrate leukotic cells in the peripheral blood even when the white blood count has not been increased.

For that matter, the classification of the cases into leukemic and aleukemic is here rather worthless, as previous investigations (*Furth*, 2) and my own experiences have shown that as a rule the cell count does not increase till shortly before the »spontaneous« death of the mouse. In most of the present experiments I have not waited to kill the animals till they became moribund, but they have been picked out when they showed large peripheral lymph nodes or marked enlargement of the liver and spleen. Thus we cannot exclude the possibility that the animals might have shown a higher white blood count if they had lived longer. In 12 cases the pathological cells in the blood and in the infiltrations were found to consist exclusively of cells of the character described above. These cases must be designated as stem-cell leukosis.

In 2 cases, Aka 124 and Aka 25, the blood presented, besides the stem-cells mentioned, also a number of cells which plainly form a continuous transition to normal large and small lymphocytes. The pathological lymphocytes are characterized by a far greater variation in their size than normal, and the protoplasm is relatively strongly basophil. In addition, also many large lymphocytes were seen to have a lobated nucleus. This corresponds quite to the picture of lymphatic leukosis in mice described by *Furth* (3).

Table 1.

The Spontaneous Cases employed for Transplantation.

A.	23	Aleukemic stem-cell leukosis.		
A.	37	»	»	»
A.	14	»	»	»
A.	19	»	»	»
A.	13	»	»	»
A.	11	Leukemic	»	»
A.	42	Aleukemic	»	»
A.	41	»	»	»
Aka	25	Leukemic lymphatic leukosis.		
Aka	49	Aleukemic stem-cell leukosis.		
Aka	33	»	»	»
A.	21	»	»	»
Aka	23	»	»	»
Aka	124	»	lymphatic leukosis.	

The mice designated as A as well as those designated as Aka are pure inbred Aka mice. This somewhat confusing designation was adopted with a view to the registration.

Technique of Transplantation.

The material employed for transplantation has consisted in leukotic lymph nodes, spleen and thymus tumor, besides pieces of the liver when this organ was greatly enlarged. By means of scissors the material is minced; then it is crushed cautiously in a mortar. Then Ringer's solution is added to the ground material, so that a suspension of leukotic cells is formed. Now the transplantation is performed intraperitoneally with a Record syringe and a fairly coarse needle. Each animal is given about 0.5 cc. of the suspension, which corresponds to about 0.2 cc. of leukotic cells. The intraperitoneal transplantation was adopted because it is far more favorable than the subcutaneous as to the distribution of the leukotic cells (*Furth*, 3). Of course, a wider distribution is obtained by intravenous injection, but the technique of the latter is considerably more difficult and also more time-consuming. The transplantation is performed with sterile instruments under aseptic and antiseptic conditions.

Retransplantation has not been employed, as it seemed preferable to run the risk of the slight experimental error that sometimes may result from failing takes in spite of susceptibility. For it has been proved on employment of a very small dose that it is possible to immunize susceptible animals against a subsequent larger dose; and hence it is conceivable that animals which through some mishap receive too small a dose may become immune against a later transplantation. Naturally this implies quite uncontrollable conditions. Besides, in the present experiments I have used such a large dose of leukotic cells that the possibility of failing takes in spite of susceptibility ought to be very slight (*MacDowell, Taylor & Potter*, 11).

In each transplantation experiment several litters were picked out from the aforementioned strains obtained by the Aka-B crossing, which are designated as LB mice. In addition, in every experiment also some Aka mice were transplanted for control. Further, in several experiments some pure B mice were also employed for control. Altogether 42 B mice were transplanted — without any positive take.

At the time of the transplantation all the mice employed were from 2 to 4 months old.

Two of the transplantation lines have been carried through several passages. Thus, from the spontaneous case A 11 a line was formed that was carried through 10 passages, while Aka 124 was carried through 6 passages. One line (A 37) was carried further once. When these lines were carried through several passages, no essential changes were seen in the pathologic-anatomical pictures, nor did these experiments offer any evidence of a rise in virulence — apart from a decrease in the period of survival after the transplantation, from about 20 days to about 10.

Transplantation was performed on a total of 134 Aka mice (see Table 4). In all the cases a picture developed that greatly reminded of spontaneous leukosis in Aka mice. Thus enlargement was found of peripheral lymph nodes in a somewhat varying degree, though always with distinct swelling of the nodes. As in the spontaneous cases, the mesentery always presented a rather markedly enlarged lymph node. With a few lines this lymph node was even particularly large (0.5×2 cm.) but always well defined from the surroundings, leaving the impression that the changes were localized to the lymph node itself. The spleen was always enlarged though in varying degrees; and the same applies to the liver.

In the thorax as a rule there was enlargement of the thymus and mediastinal lymph nodes. Mice transplanted with the same line showed perfectly identical pictures, whereas the different lines gave some variation in the picture — though not more than all the cases had to be taken as representatives of the same type: a picture which in its features corresponds to generalized leukosis in spontaneous cases. The few variations encountered involved chiefly quantitative differences with regard to the enlargement of the lymph nodes, liver, spleen and thymus.

On microscopic examination, just as in spontaneous, a diffuse infiltration was seen of the lymph nodes. In the smallest nodes remnants of the original lymphoid tissue were still seen. Imprint-specimens show distinctly how the leukotic cells crowd out the normal lymphocytes which are seen scattered among the former. Specimens of the liver show pronounced perivascular and diffuse infiltration. The spleen is diffusely infiltrated; in most of the cases the normal structure has been effaced completely. The kidney shows most often a very pronounced perivascular infiltration round the large vessels in the hilus, but infiltration is encountered in the cortex too — here in the form of accumulation of leukotic cells in the glomeruli and round the tubules. The thymus shows diffuse infiltration. In some cases where the enlargement of the thymus is very marked the lungs present

a rather pronounced degree of perivascular and peribronchial infiltration.

The cell type is the same as encountered in the spontaneous cases. The peripheral blood contains a somewhat varying number of cells. In most of the cases, however, the leukosis has to be considered as aleukemic — but, as mentioned already, the rise in the cell count does not appear till the last stage of the disease. Still, a good deal of leukotic cells are always seen in the blood stream. There appears not to be any definite connection between the white blood count in the spontaneous cases and the corresponding count in the transplanted cases. On the other hand, there is found to be a certain uniformity in the percentage of leukotic cells in the blood in the various lines. As far as that goes, I have found the same features as described by *Furth* (1): that the percentage of immature cells is proportional to the white blood count, which rises in the last phase of the disease.

In transplantation of the crossing LB mice we find in the 2' filial generation a percentage of takes amounting to 44.2 % (19 positive among 43 transplanted animals, cf. Table 5). From the first filial generation so far only a very limited number of animals have been transplanted, on which account we have not yet sufficiently reliable figures on the susceptibility or information about the pathologic-anatomical picture.

Further, as seen from Table 5, transplantations were performed on the aforementioned inbred strains from the 2' filial generation. The litters were picked out at random from the generations that have appeared so far. Altogether 324 LB mice were transplanted with a total number of positive takes amounting to 128, i. e., 39.5 % of the transplanted animals.

The figures here obtained are yet too small for reliable calculations on the heredity of the susceptibility to transplanted leukosis. The greatest interest in these experiments is attached to the finding that the pathological changes in the LB mice show a very considerable variation in comparison to the Aka mice. In a great majority of cases we meet with a picture which completely resembles the picture previously described in transplantation on Aka mice (cf. p. 17).

In 14.8 % of the takes (19 out of 128), however, instead of generalized leukosis I found a large tumor formation retroperitoneally and in the mesentery. Here there was a macroscopically pronounced infiltrative growth, especially round the genitals. The liver and spleen were normal in size; but in a few cases where the tumor was adjacent to the spleen, this organ was also infiltrated and somewhat enlarged. There was no enlargement of the peripheral lymph nodes. In most of these cases the thymus and mediastinal lymph nodes looked normal, but enlargement of these structures was seen in a couple of cases.

The tumor was of the same grayish-white color as the leukotic

lymph nodes in the animals with the generalized form. No areas of necrosis were seen. The tumor consisted of the usual leukotic cells of the same appearance as in the generalized cases. The stroma is very scanty with few blood vessels. In specimens fixed ad modum Helly and stained with ordinary hematoxylin-eosin no difference can be made out between the leukotic cells here and those seen in generalized cases. The picture seen when lines of lymphogenous leukemia were used may be compared to that of lymphosarcoma in spontaneous cases or perhaps rather to lymphosarcomatosis, at any rate in those cases where the changes involve also the mediastinal lymph nodes. In the other cases the designation of the picture has to be limited to »leukotic tumor«.

Microscopic examination of the organs shows in the liver an exceedingly scanty infiltration with leukotic cells as compared to the usual findings in the generalized cases; most often this infiltration involves merely a few perivascular cells. In cases without macroscopic enlargement of the spleen this organ presents a perfectly preserved follicular pattern. As the kidney lies in close proximity to the tumor, this organ shows some hilar infiltration but no diffuse infiltration. Specimens of the blood were not taken in every instance on autopsy, as it seems desirable also to estimate the period of survival of the animals after the transplantation; so some of the mice were allowed to die spontaneously.

In the several cases examined, however, the white blood count was low, and in 4 of these cases no pathological cells were found. In 3 cases a few leukotic cells were seen, as shown in Table 2.

Table 2.

White Blood Count in the Transplanted Animals in Relation to the Type of Takes.

Type of take	Experimental animals	White blood count	% of leukotic cells (stem-cells or lymphoblasts)
Generalized leukosis	Aka (39 animals)	34,880 (2500—257,000)	34.3 % (0—90 %)
Generalized leukosis	LB (19 animals)	30,800 (4500—83,000)	36.8 % (2—100 %)
Tumor type	LB (7 animals)	11,500 (7400—17,300)	1.3 % (0—4 %)

In 14.1 % of the cases (18 out of 128 positive, cf. Table 4) we found a picture which appeared rather as an intermediate type between the generalized cases and the tumor type. The same tumor formation is seen in the abdomen, with infiltrative growth, as encountered in the localized tumor type; at the same time, however, there is universal enlargement of lymph nodes and also enlargement of the liver and spleen. Microscopy of the various organs showed

the same picture as seen in the generalized cases, though perhaps a little less advanced.

Another characteristic feature of these types is the period of survival, *i. e.*, the number of days the mouse lives after the transplantation till it dies spontaneously or is so ill that one has to kill it in order to make sure of good microscopic preparations and specimens of blood for examination.

From Table 3 it will be noticed that the period of survival for animals with the tumor type on an average is much longer than for the two other types that have nearly the same period of survival.

Table 3.

Average Age at the Time of Transplantation and Average Period of Survival in Relation to the Type of Takes.

Strains of animals	Type of take	Average age at transplantation	Average period of survival
134 Aka	Generalized leukosis	85.1 days	15.8 days
91 LB mice	Generalized leukosis	94 days	18.6 days
18 LB mice	Intermediate type	91.1 days	21.5 days
19 LB mice	Tumor type	88.3 days	36.7 days

Whether the considerably longer survival signifies that the cells in this type are less malignant will still have to be left unsettled at this stage of the investigation. But, among others, one has to consider the possibility that with the entirely local growth of the lesion the leukotic cells spare the vital organs (liver, lungs and bone marrow) thus prolonging the lifetime of the animal.

In 7 of the tumor cases the bone marrow was examined by means of imprint-specimens stained after Giemsa, without showing any sign of infiltration with leukotic cells. These examinations were estimated by comparison with normal bone marrow from mice.

In the generalized cases, in all the cases examined, the bone marrow was seen to show some infiltration — in a few cases even complete suppression of all normal elements in the marrow. One might characterize the generalized cases by saying that here the leukotic cells grow in the organs and tissues which include the reticulo-endothelial system, whereas in the localized forms the leukotic cells grow preferably locally — at the site of transplantation.

From Table 4 it will be noticed that the tumor cases are distributed fairly equally over the various experiments and lines. Thus it would hardly be practicable to point out a line with any particularly great frequency of tumor cases.

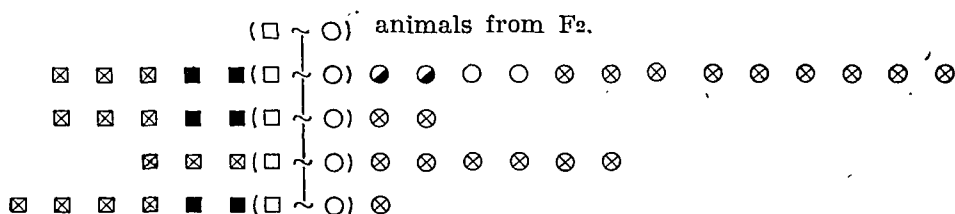
Table 4.

Distribution of Susceptible LB Mice after the Various Types of Takes in the Various Experiments.

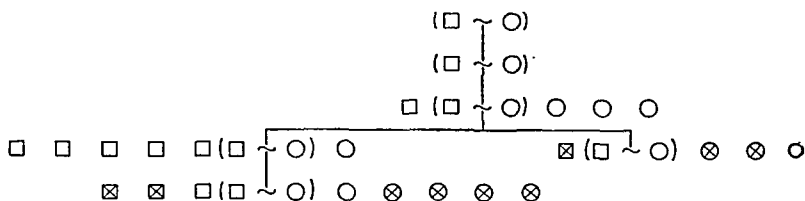
For control the Aka mice transplanted simultaneously are recorded in the columns to the right.

Exp. No.	Passage	Donor	No. of transplanted LB mice	No. of positive takes	Generalized leukosis	Intermediate type	Tumor type	No. of transplanted Aka mice	No. of positive takes	Generalized leukosis
1	1	A 23	0	—	—	—	—	14	14	14
2	1	A 37	7	2	2	0	0	5	5	5
3	2	Aka 63	4	1	1	0	0	9	9	9
4	1	A 14	7	6	2	2	2	7	7	7
5	1	A 19	15	11	8	0	3	4	4	4
6	1	A 13	9	4	1	2	1	12	12	12
7	1	A 11	8	0	—	—	—	3	3	3
8	2	Aka 99	12	7	3	1	3	4	4	4
9	3	Aka 234	9	7	5	2	0	3	3	3
10	4	Aka 244	14	3	1	1	1	2	2	2
11	5	Aka 255	5	2	0	0	2	3	3	3
12	6	Aka 280	0	—	—	—	—	7	7	7
13	7	Aka 273	0	—	—	—	—	3	3	3
14	7	Aka 277	2	2	0	2	0	0	—	—
15	8	Aka 300	4	4	4	0	0	5	5	5
16	9	Aka 297	3	3	3	0	0	3	3	3
17	9	Aka 296	5	0	0	0	0	0	—	—
18	9	Aka 295	12	11	11	0	0	0	—	—
19	10	Aka 261	3	0	0	0	0	5	5	5
20	1	A 42	13	12	11	1	0	4	4	4
21	1	Aka 25	14	4	2	1	1	0	—	—
22	1	Aka 49	9	0	0	0	0	4	4	4
23	1	A 21	17	10	10	0	0	6	6	6
24	1	Aka 23	15	3	3	0	0	2	2	2
25	1	A 41	15	0	0	0	0	0	—	—
26	1	Aka 33	21	14	14	0	0	3	3	3
27	1	Aka 124	13	2	2	0	0	5	5	5
28	2	Aka 290	4	2	1	0	1	4	4	4
29	2	Aka 288	7	2	0	0	2	0	—	—
30	3	Aka 310	14	0	0	0	0	6	6	6
31	3	Aka 311	18	4	1	3	0	0	—	—
32	4	Aka 319	6	0	0	0	0	7	7	7
33	4	Aka 318	18	3	2	0	1	0	—	—
34	5	Aka 377	10	9	4	3	2	4	4	4
35	5	Aka 374	7	0	0	0	0	0	—	—
36	6	Aka 392	4	0	0	0	0	2	2	2
		Total	324	128	91	18	19	134	134	134

In Table 5 the transplanted LB mice are arranged after their relationship, the mice of the 2' filial generation and the above-mentioned strains being arranged separately. From this it will be noticed that the percentage of takes varies greatly from one strain to another — as was also to be expected from the way in which the various strains are formed, as animals of most different genetic constitutions are picked out from the 2' filial generation. Thus strains X and XIX appear to be perfectly resistant while strains IV, VI and VIII appear very susceptible. Further, it will be noticed that the tumor cases accumulate in a few strains, VII and XVI. But the following pedigrees show even more plainly the difference between the individual strains — a difference that cannot be explained as accidental alone but has to be looked upon as depending on genetic differences between the strains.



Strain XVI.



Strain VI.

This pedigree includes the outcome of more recent experiments that are not recorded in Table 5.

- = generalized type.
- ◼ = intermediate type.
- = tumor type.
- ⊗ = negative take.

The pairs recorded in parentheses are non-transplanted breeding animals.

Thus it appears that also genetic factors in the host determine how the leukotic cells grow in the transplanted organism. From the

figures recorded here it is not practicable to decide on the finer mechanism of the inheritance, but it may be that further investigations perhaps will be able to reveal such figures as may be brought to comply with the known genetic laws.

Table 5.

Strain	No. of trans-planted animals	Positive	Generalized	Generalized + tumor	Mainly tumor
2' filial generation	43	19	10	6	3
I	20	4	4	0	0
II	35	9	7	0	2
IV	7	7	7	0	0
VI	11	10	10	0	0
VII	10	4	1	0	3
VIII	17	15	12	1	2
X	20	0	0	0	0
XII	29	12	8	3	1
XIII	4	0	0	0	0
XV	10	4	4	0	0
XVI	41	10	2	2	6
XIX	9	0	0	0	0
XX	10	2	1	1	0
XXI	11	3	2	0	1
XX	10	9	9	0	0
XXI	24	12	8	4	0
XXVII	7	5	5	0	0
XXVIII	6	3	1	1	1
Total	324	128	91	18	19

It seems obvious to parallelize the findings here recorded with the natural variation observed in the spontaneous instances of leucosis and their connection with the corresponding tumor forms, as one might readily imagine that also in the spontaneous cases it would be genetic factors that determine the development of the type. Such a conclusion is not justifiable, however, as undoubtedly there is an essential difference between the spontaneous leukoses and the morbid features resulting from transplantation, and yet it may not be excluded that it is a single phenomenon like this, for instance, which in both cases are influenced by the same factors.

In the strains here described with a tumor-like take, we have at any rate an object for study that possibly may lead to some elucidation of the conditions concerning the growth of the leukotic cells in the organism.

Summary.

After review of the literature on transplantation of leukosis in animals, with a special view to variation in the pathologic-anatomical picture, a description is given of the preliminary results of transplantation experiments on animals obtained from crossing between two mice strains, Aka and B, respectively with a high and a low frequency of spontaneous leukosis. The material for the transplantations is leukotic tissue from spontaneous cases in the Aka strain.

In the Aka mice only one type of take is seen, and it is comparable to generalized leukosis.

In animals of the crossing Aka \times B in the second filial generation and in strains formed by inbreeding from this generation, a rather considerable variation is seen in the picture of the takes. The pathologic-anatomical findings are divided into three groups: 1) generalized cases, 2) tumor-like take + generalization, and 3) preponderantly tumor-like take.

It is pointed out that the distribution of the findings on the different strains makes it rather likely that the genetic factors in the hosts determine the different outcome of the transplantation.

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Fig. 2.
LB-mouse with growth of the transplant com-
parable with generalized leukosis.



Fig. 1.
LB-mouse with tumorlike growth of the trans-
plant (lymphosarcomatosis).



Fig. 3.

Aka-mouse with a take of the same type as Fig. 2.

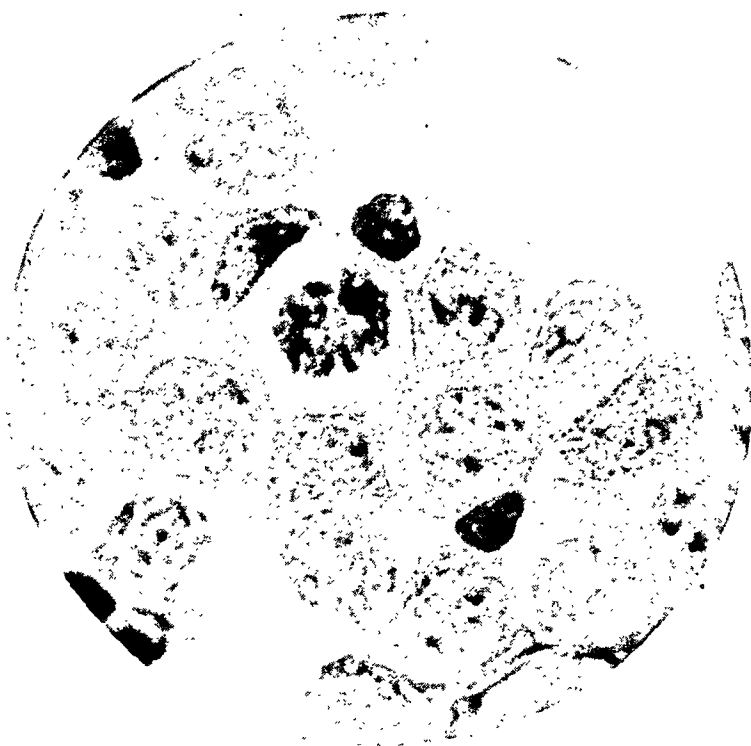


Fig. 4.

Imprint-specimen of lymph node from Aka-mouse with transplanted leukemia. The leukemic cells are seen, one in mitotic division. Three normal lymphocytes are seen between the leukemic cells. $\times 1400$.

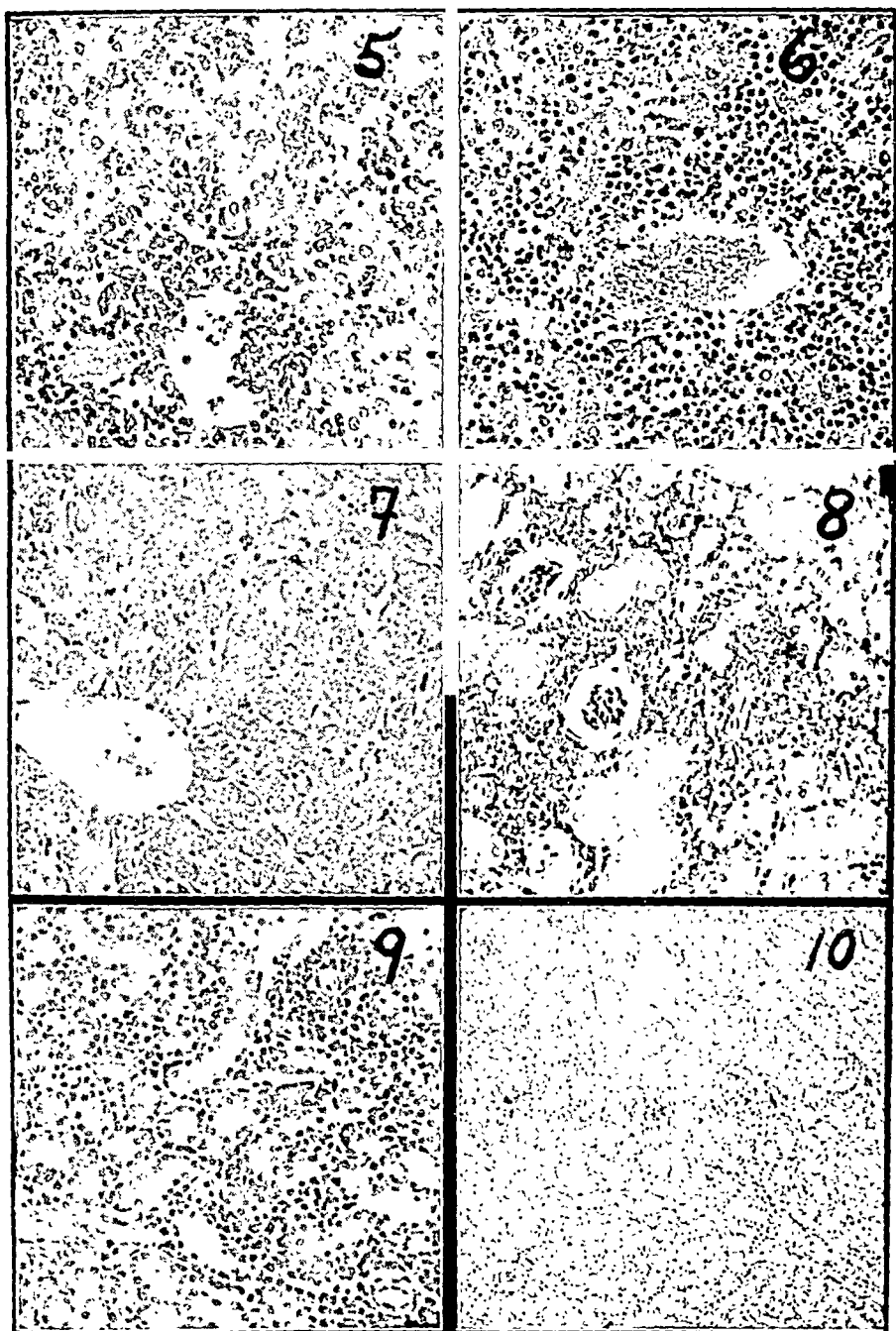


Fig. 5: Liver of inoculated Aka-mouse. Extensive infiltration is seen. $\times 240$. — Fig. 6: Liver of LB-mouse with generalized growth of the transplant. The lesions are identical with the lesions of the Aka-mouse. $\times 240$. — Fig. 7: Liver of LB-mouse with tumor-like take. Practically no infiltrations are seen in the liver. $\times 240$. — Figs. 8, 9 & 10: Kidneys of an Aka-mouse and two LB-mice respectively. The former LB-mouse with generalized growth of the transplant, the last with tumor-like growth. Figs. 8 and 9 show perivascular and diffuse infiltration. Fig. 10 shows no infiltration. Figs. 8 and 9 $\times 240$. Fig. 10 $\times 96$.

DANSK PATOLOGFORENING 11. MØDE 15/3 1944 I KØBENHAVN.

Eleventh Meeting of the Danish Pathological Society, March 3, 1944.

Onzième séance des pathologistes danois le 3 mars 1944.

Elfte dänische Pathologentagung am 3. März 1944.

Poul Emsbo: *Ossification of the Aorta and Pulmonary Artery in a Heifer.*

Autopsy on a heifer, 2 years old, which died suddenly, revealed that the thoracic aorta (for a distance of about 25 cm. from the heart), the main stem of the brachiocephalic trunk, and the pulmonary artery had undergone ossifications in the wall to such an extent that they were transformed into rigid tubes. The heifer had died from rupture of the aorta (fracture) followed by hemorrhage into the left pleural cavity.

On histological examination, a bone plate (bone tube) was found chiefly to consist of compact osseous tissue with typical lamellar structure embedded between the intima and media. At the heart the wall of the bone tube measured 2—3 mm. in thickness, in the arch over the aorta it was decreasing to 1 mm., whereafter it became thin as paper. In the thickest part of the tube, a smaller area was found to consist of spongy bone tissue, in the spaces of which a cellular reticulum (bone marrow?) was demonstrated. Irregular spaces in the bone tube were found to be empty or partially filled with fat (fatty marrow).

Intima: Sclerotic, varying greatly in thickness, with reduction in the amount of muscular and elastic elements. More deeply, here and there areas of fat infiltration in the meshes of the connective tissue, together with the formation of fat-containing detritus and dystrophic calcification. Media: Atrophy of the muscle bundles; relative increase in connective tissue, together with the formation of fibrous connective tissue in spots or streaks or appearing as a periosteum-like layer on the bone plate. In large areas the elastic lamellae were the site of swelling and extensive vacuolization in varying degrees, in some places with complete dissolution (analogous to medionecrosis idiopathica — Erdheim) and with fragmentary and granular decomposition adjacent to the bone plate (Dimitrijeff). In addition, there were scattered small areas of calcification. In the depth of the media, diffuse infiltration with round-cells, plasma cells and granulocytes, among which eosinophils were so numerous as to give a picture of pronounced eosinophilia. In the media and adventitia, in the surroundings of the fracture, extensive infiltrating hemorrhages were demonstrated.

As to the etiology and pathogenesis of the ossifications, no definite conclusions could be drawn. From the location and progression of the ossification, the process presumably was to be characterized as ossification of the media, but histological details might indicate the intima as the origin of the ossification. The bone formation took place as direct ossification, by transformation and apposition of bone tissue, especially from the connective tissue of the media.

As to the cause of the aorta fracture, it seems conceivable that excessive arching of the vertebral column or the blood pressure effect in connection with the degenerative changes in the non-ossified layer of the aortic wall may have brought about the fracture.

From Uruguay, Rubino has described a case which fundamentally is in keeping with the one here described.

A more detailed account of the case, with 1 figure and 7 microphotos, is published in *Maanedsskrift for Dyr læger*, vol. 56, p. 94, 1944.

No discussion.

Sven J. Olsen: *Infection with Group G Streptococci in Animals.*

A brief review is given of the investigations reported by Lancefield & Hare in 1935 and by Hare & Fry in 1938.

Human Group G streptococci appear to be relatively harmless bacteria, causing no fatal pathological condition in man. In animals, however, infections with these bacteria seem to behave differently.

Two G strains were isolated from extensive purulent processes in 2 cats. In 11 cases of distemper pneumonia in dogs, the material was found to contain Group G streptococci in 5 cases. In throat cultures from 6 apparently healthy dogs the presence of Group G infection was demonstrated in 1; and from 6 dogs suffering from distemper at the catarrhal stage, Group G streptococci were isolated in 5 cases.

An interesting case of puerperal infection in a Pekingese dog, resulting in fatal septicemia in the pups, was investigated more thoroughly. Group G streptococci were isolated from the vaginal discharge of the mother and from the blood, spleen and liver of the pups.

Altogether 19 animal G strains were examined besides, for comparative tests, 11 human G strains. All the strains were examined with respect to their cultural, biochemical, serological and fibrinolytic properties. On blood agar all the strains produced typical β -hemolysis. Generally the human strains showed a larger diameter of the zone of hemolysis than did the animal strains. The average diameter for the 11 human strains was 3.36 mm. (extreme values, 3.00 and 3.75). For the 19 animal strains it was 2.50 mm. (extreme values, 1.50 and 2.25). Biochemically the 10 human strains were found to ferment trehalose, glucose, lactose and saccharose, but not sorbite, mannite, inulin and raffinose. One human strain, on the other hand, was lactose-negative.

Of the animal strains 3 presented the same fermentative properties as the human strains, whereas 5 animal strains behave like the one human strain. On the other hand, 11 animal strains differed from the 10 human in being trehalose-negative. None of the strains broke down hippurate. The end pH in 1 % glucose broth was about 5 (extreme values 4.65 and 5.25).

Serologically all the strains gave precipitation with Group G serum, not with A, B, C, E, H and K sera.

All the human strains dissolved human fibrin in fibrinolysis tests, while none of the animal strains was capable of this.

An attempt to obtain the reverse result by repeating the fibrinolysis tests with dog fibrin was not successful as neither human nor animal strains

dissolved dog fibrin. These experiments were merely preliminary, however. Future experiments will have to decide whether fibrinolysis tests may afford a measure for differentiation between animal and human G strains — just like in the case of the trehalose-positive, sorbit-negative Group C streptococci. The strains here examined were strongly pathogenic for mice.

No discussion.

H. E. Ottosen: *Some Pathologic-anatomical Observations on Avian Tuberculosis in Cattle.*

Description is given of 5 cases of avian tuberculosis in cattle in which the lesions presented either an extraordinarily wide distribution of the infection, an uncommon localization, or particularly severe local changes.

Case 1 showed hematogenous, nodose, caseated processes with secondary involvement of the small bronchi, besides productive pleural and peritoneal tuberculosis.

In Case 2 a calf had an avian infection in lungs which beforehand were the site of dense catarrhal infiltration.

In Case 3 a heifer presented ulcerative intestinal tuberculosis and diffuse caseation of the mesenteric and retropharyngeal lymph glands.

Case 4 concerns a cow with miliary tuberculosis of the lungs and productive tuberculous processes in the pulmonary lymph glands and kidneys, besides pleural and peritoneal tuberculosis.

In Case 5 a cow aborted on account of infection with avian tubercle bacilli in the uterus and membranes. The cow was slaughtered 3 weeks later. Now examination revealed markedly productive, mostly unspecific, new-formations in the uterine mucosa, on the peritoneum, in the udder, mammary lymph glands and deep inguinal lymph glands. — The pathogenesis of the lesions in this case is elucidated fairly well, as naturally it must have been the extremely massive infection of the uterus which has spread so wide about in the organism through the blood vessels and the lymphatics. On the whole, the frequent infections of the pregnant uterus are taken to play a considerable role as the starting-point for the further spreading of the infection, in particular when the peritoneum is involved, for, as in bovine tuberculosis, the spreading may readily take place through the Fallopian tubes to the peritoneal cavity.

In all five cases avian tubercle bacilli were isolated from one or more of the organs attacked. The strain obtained in Case 5 was somewhat atypical in its growth, however, as it in certain respects reminded of the bovine type. In appraisal of its virulence on guinea-pigs and rabbits it was found to be a little more virulent than are avian strains in general.

Discussion:

H. C. Bendixen: It is of considerable interest to see that the avian infections may bring about such marked pathologic-anatomical changes in cattle.

When Ottosen has been able in a spontaneously infected cow to demonstrate tuberculous changes in the udder tissue produced by avian tubercle bacilli — even though they are atypical — the old question about the possibility of the excretion of such bacilli with the milk arises again. Although attempts to demonstrate such excretion of tubercle bacilli hitherto have turned out negative, there still is every reason to continue these attempts. For the possibility of such excretion has been demonstrated indeed by Griffith in an experimentally infected goat.

As to the pathogenesis of the lesion in this case, to me it seems the most natural explanation that the udder of this cow has become infected through the same bacteriemia which originally and primarily infected the uterus.

L. Heerup: Wherefrom and how are the ruminants infected with avian tuberculosis?

Aage Jepsen called attention to the fact that one of the avian strains apparently was atypical and asked whether more thorough studies had been carried out on the cultural properties and virulence of the strains concerned. It seemed conceivable that the avian type of the tubercle bacillus might include some special varieties that are more virulent for mammals than is the ordinary avian tubercle bacillus isolated from birds.

H. E. Ottosen: To Professor Bendixen I wish to say that for the present, I think, we shall have to leave it an open question whether avian tubercle bacilli may be excreted with the milk, in particular in the case of cows which have aborted on account of avian tuberculous infection of the uterus. But it is our intention in a near future to try to carry through a thorough examination of the milk from such animals.

As to the point of time for the spreading of the avian infection in the organism, I shall willingly admit that theoretically it is possible that this process may have taken place simultaneously with the infection of the uterus, which naturally must be of hematogenous origin too. But to me it seems more reasonable to assume that it has taken place in connection with the abortion, as at this time there has been an enormous depot of tubercle bacilli in the uterus, and at the same time the resistance of the organism has been lowered to some extent. Indeed, a bacteriemia giving rise to infection of the uterus need not be particularly pronounced, as the pregnant uterus presents a locus minoris resistentiae. In this connection it will be appropriate to remind of the fact that, in spite of apparently complete recovery after the abortion, the infection may persist in a latent form to the next pregnancy and then again give rise to abortion.

At all events, I think that at any rate in the last of the cases here reported it is justifiable from the morphological character of the changes to conclude that they are of a quite recent origin.

To Dr. Heerup I should like to say infection of cattle with avian tuberculosis naturally, as a rule, comes from tuberculous poultry, especially hens, but also other birds are to be taken into consideration — for instance, pheasants and sparrows. The infection takes place through ingestion by the cattle of the droppings from the tuberculous birds. The cows do not infect each other.

As to the question raised by Professor Jepsen — about the occurrence of atypical strains of the avian tubercle bacillus — I quite agree, of course, that such strains may be encountered now and then. In this connection I may mention that in the State Veterinary Serum Laboratory we just now are determining the virulence of a strain which in its growth reminds of the bovine type, while in its antigenic properties and its virulence for cattle it comes nearer the avian type.

N. O. Christensen: *Filariasis and Microfilariasis in the Horse.*

By Filariasis and Microfilariasis are meant invasive diseases caused by nematodes of the order of filaria and their larvae, the microfilaria.

These diseases are known and have especially been studied in man and

in dog. Blood microfilariasis in European horses was first described by Wirth in 1911 from Austria-Hungary and has since been shown to be present in several other countries, as in Italy, Germany, Sweden.

Through the investigations of Wirth and others it can be regarded as proved that microfilaria provided with sheaths in European horses are derived from *Setaria equina*, a filiform thin white nematode, living in the abdominal cavity of the horse.

The demonstration of the microfilaria which occur in the circulating blood is most conveniently carried out by the method devised by Nordstrom by which $\frac{1}{2}$ cc. of blood is hemolyzed in 10 cc. of distilled water after which it is centrifuged; in drop preparations of the slimy sediment the microfilaria are easily demonstrated in positive cases.

The microfilaria measure $5-7 \times 250-290 \mu$. In appearance they are thin extremely mobile worms, tapering much at the posterior end. Round the worm there is a sheath which is assumed to be the much elongated egg-shell.

In stained preparations various characteristic organs or the primordia of such may be shown in the inside of the nematode.

Of horses admitted to the medical clinic of the Royal Veterinary and Agricultural College, 201 in all have been examined. Among these there were 17 positive cases of microfilariasis, that is to say, 8.5 % of the animals examined.

The age of the animals affected was chiefly between 1 and 6 years. There were in addition some few older horses, thus a 13-year-old.

Microfilariasis occurred principally in the months of March-September. In the other months of the year no microfilariasis was found in the material of the clinic.

The number of microfilaria ranged from quite to 1750 per cc. of blood. Generally the number was below 100 per cc. of blood. It was not possible to demonstrate any periodicity.

In addition, 72 thorough-bred horses from various racing stables were examined 12 of these, or 16 %, showed microfilaria.

Within the various age-classes among these horses microfilariasis was of the following frequency: less than 1 year 0; 1 year 25 %; 2 years 37 %; 3 years 50 %; 4 years 50 %; and 6 years 40 %. Microfilariasis has not been found in horses over 6 years old in this material.

Two horses were kept under observation for about a year. In this period there were still microfilaria in the blood in varying numbers, the peak being in June-July.

According to these investigations microfilariasis does not seem to have had any pathogenic significance.

No discussion.

H. C. Bendixen: *Littery Occurrence of Anophthalmia or Microphthalmia together with other Malformations in Swine — Presumably due to Vitamin A Deficiency of the Maternal Diet in the first Stage of Pregnancy and the Preceding Period.*

Description is given of anatomical changes found in littery anophthalmia or microphthalmia in swine. Usually the condition is accompanied by a complex of other inhibitory malformations. Observation on cases encountered in Denmark indicates that the affection is due to vitamin A deficiency of the maternal diet. Will be published in extenso in *Acta pathologica et microbiologica Scandinavica*, Supplement LIV — 1944.

Discussion.

A. Møller-Sørensen: Demonstration of photos of a calf coming from a »first-class« herd and showing: Hypoplasia of the eye-ball, internal hydrocephalus (slight degree), pleosomia e, rectovaginal fistula, spastic paresis of the hind legs (Little).

Tage Kemp: In man too we sometimes meet with fetuses or newborn presenting a combination of microphthalmia or anophthalmia with hare-lip and cleft palate. In such cases it will perhaps be appropriate also to consider the possibility of an avitaminosis in the mother.

DANSK PATOLOGFORENINGS 12. MØDE 10/5 1944 I KØBENHAVN.

Twelfth Meeting of the Danish Pathological Society, May 10, 1944.

Douzième séance des pathologistes danois le 10 mai 1944.

Zwölfte dänische Pathologentagung am 10. Mai 1944.

Gunnar Teilum: *Homologous Tumors in the Ovary and Testes. (Contribution to Classification of the Gonadal Tumors).*

The reader is referred to the previous papers of the author in *Beiträge z. path. Anat.* 108, 534, 1943, *Nordisk Medicin* 20, 2305, 1943 and *Acta obstetricia et gynaecol. Scandinav.* 1944.

For elucidation of the histogenetic aspects of the gonadal tumors as the basis for a more elaborate classification of the tumors in keeping with biological or clinical characteristics, the demonstration of *homologous* tumors in the ovary and testis is of particular importance. Of such tumors, so far only a very few are known, above all the seminoma (dysgerminoma). In the ovary this tumor would originate from persistent remnants of the medullary cords, which are homologous with the early stages of the germ-cells in the testis. Also the primary gonadal chorionepithelioma may occur in the testis as well as in the ovary. Hitherto, however, this form of tumor was generally classified somewhat differently in the two organs. Accordingly, chorionepithelioma of the testis was regarded by Ahlström (1931) as a special form of *testicular* tumor. In the ovary on the other hand, the corresponding tumor is nearly always classified as belonging to the teratomas — for instance also by Novak (1940).

In 1943, however, the author has set up a homologous tumor series (the »seminoma series«) in the testis and ovary after demonstration of the homology (with total morphological congruence) of an ovarian tumor — which previously has been misinterpreted as mesonephroma (Schiller, 1939) and papillo-endothelioma (Kazancigil, Laquer & Ladewig, 1940) and a most often adenopapilliferous, solid or cystic, possibly teratoid testicular tumor. On the basis of its relation to seminoma and chorionepithelioma, besides its clinical and hormonal aspects, I have now classified these in testicular and ovarian morphologically well-defined tumors as an intermediate form be-

tween seminoma (dysgerminoma) and chorionepithelioma and designated the tumor as »*the intermediate form of the seminoma series*».

As pointed out by Robert Meyer (1930) in connection with the arrhenoblastoma, the decisive point is always to demonstrate the connection between the individual tumor forms, notwithstanding their deviations due to different degrees of differentiation. The same applies to the granulosa-cell tumors and also to the tumors we are dealing with here designated as the seminoma series (gonocytoma), which are taken to arise from the early stages of germ cells in the testis or persistent remnants of the medullary cords in the ovary.

Meyer emphasizes that the potential capacity of these undifferentiated cells in their anlage is not known, and he assumes that to begin with they lost the capacity for differentiation into specific germ cells. The transitions described through an intermediate (adenopapilliferous) form to syncytial chorionic epitheliomatous proliferation with corresponding excretion of chorionic hormone, besides the aforementioned relation to the polycystic teratoma, are suggestive of a tetipotent cellular origin, so that the development of the chorionepithelioma has to be looked upon as parthenogenetically arising from such undifferentiated totipotent cells of the germ-cell series. As these cells in the *ovary* normally undergo retrogression, so that in most cases they leave no trace whatever, while in the *testis* they correspond to the earlier stages of the germ cells, we have in this way a natural explanation of the far more infrequent occurrence of the primary gonadal chorionepithelioma in the ovary than in the testis, with or without relation to teratoma. Viewed in this way, the origin of the chorionepithelioma in the ovary too will be a special *testicular* anlage.

The decisive point is that the chorionepitheliomatous proliferations in all cases — in the ovary as well as in the testis, and also when they occur together with the polycystic testis teratoma — have their origin in this particular cell material, the dominance of which in the male gonads is due to the greater frequency of these tumors in the testis than in the ovary; and this applies to *all three tumor forms*: seminoma, intermediate form, and chorionepithelioma. It further explains why the occurrence of these tumors in the ovary are limited to the younger age-classes in a much higher degree than in the testis and why in women these tumors more often are associated with developmental anomalies in the genital system — *e. g.* intersexuality, virilism.

The intermediate form of the seminoma series was previously regarded, for instance, as perithelioma, endothelioma, adenocarcinoma, angiosarcoma, Wolffian epithelioma. Occasionally, when situated in the ovary, the tumor has been misinterpreted as granulosa-cell tumor. In Bang's classification of the testicular tumors the intermediate form will belong to the group called epithelioma mixtum syncytiomatodes, to which group Bang also reckons the chorionepithelioma. Here, then, it does not appear as a morphologically well-defined tumor and consequently in its pure form it will be mistaken for an adenocarcinoma or some other »non-specific« tumor. Furthermore, the designation »epithelioma mixtum« can only be suggestive of a mixed tumor — indeed it has been grouped together with such mixed tumors in general by other authors (cf. Grevilius, p. 24) —, whereas it is just the *continuity* between the three different forms of the same series that is to be emphasized.

In comparative studies on ovarian tumors of the arrhenoblastoma type and various testicular tumors, tumor forms are demonstrated beyond the rare tubular adenoma (Pick), which are to be looked upon as homologous. These tumor-forms are grouped together under the designation: the *androblastoma series*. In a few cases it is impracticable by morphological criteria alone to distinguish between ovarian tumors of the arrhenoblastoma type

Homologous tumors of the seminoma series (gonocytoma)		
Ovary	Testis	Secretion of chorionic hormone
Dysgerminoma	Seminoma	0
Intermediate form = Adenopapilliferous epithelioma		(+)
ovarian	testicular	
Chorionepithelioma		++
ovarian	testicular	
Homologous tumors of the androblastoma series		
Ovary		Testis
feminizing	virilizing	
mesenchymomas		
Granulosa-cell tumor	Arrhenoblastoma	Androblastoma
differentiated	differentiated (tubular)	differentiated - (tubular)
intermediate	intermediate	intermediate
diffuse	diffuse	diffuse
Lipoid-cell tumors (= variant of the above):		
Theca-cell tumor	Tumors described as	"Interstitial-cell tumor"
"folliculome lipidique"	"adrenal tumor"	
	"luteoma"	
	"masculino-ovoblastoma"	

and granulosa-cell tumors — respectively virilizing and feminizing mesenchymomas. Several tumors which previously were regarded as virilizing (defeminizing) granulosa-cell tumors are taken to be virilizing mesenchymomas

of the androblastoma series. Groups of epithelial-like, lipoid-containing, cells (»lipoid cells«) may occur in the arrhenoblastoma as well as in the homologous tumors of the testis.

Virilizing ovarian tumors of the type described as »*luteoma*«, »*adrenal tumor*« or »*masculino-ovoblastoma*« are interpreted as a special variant of the androblastoma series, arising through one-sided preponderance of the »lipoid cells« in virilizing mesenchymomas (of the arrhenoblastoma type), in analogy with the conception of the theca-cell tumors as a special form of feminizing mesenchymoma. The virilizing »*lipoid-cell tumor*« of the ovary has to be looked upon as homologous with certain forms of interstitial cell tumors of the testis.

The homologous series of tumors here set up serve as basis for a common classification of the gonadal tumors.

Discussion.

Fridtjof Bang: We have been most interested in Dr. Teilum's paper, which brings several new viewpoints, especially as far as the arrhenoblastomas are concerned. The excellent lantern slides seemed most convincing. We have all reason to thank Dr. Teilum for tackling this subject, which greatly needs further investigation and elaboration.

But I cannot agree with Dr. Teilum on every point — in particular as far as the grouping of these tumors is concerned. Dr. Teilum has attached the chief importance to comparative morphological studies. These studies meet with difficulties of various nature, however. For one thing, the tumors are often so large and variable in structure that is practically impossible to examine them through serial sections in order to be able to register all the different structures that may occur in a given tumor. As a matter of fact, a complete examination would really require also examination of all the metastases as these often may deviate from the main tumor.

It has long been a widely accepted view that the Wolffian body, the mesonephros, played an essential role in the histogenesis of these tumors. It was Chevassu, I think, who first brought about that this view was discarded and that now practically all the testicular tumors are taken to arise from the germinal epithelium. The more thoroughly we get to know these tumors, the more convinced we become that this assumption is correct — and these investigations have nowadays been aided greatly through the hormonal studies for which Dr. Christian Hamburger deserves the greatest credit. Thus, in the testis we meet with tumors, the structure of which has led to the assumption that they originated from the mesonephros, while hormonal analysis showed that they produce gonadotrophin. It would be unreasonable to take tumors of such structure — including, among others, areas which sometimes are interpreted as glomerular anlage, sometimes as fetal germinating anlage — to originate from the mesonephros. We have to assume that all these, greatly varying, tumors originate from germinative cells with the possibility for differentiation of trophoblasts and production of chorionic hormone.

In the classification of testicular tumors attempted by me some years ago, I started from such points of view and tried to base the classification more on our knowledge concerning the germinal epithelium and the development of the gonads, supplemented by Dr. Hamburger's hormonal investigations, than on examination of the structure of the individual tumors. For examination of the structure of a tumor will often lead to erroneous and undemonstrable views — as has been illustrated very well just through the correction of Schiller's findings by Dr. Teilum.

Besides the clear-cut cystic teratomas, I set up three main forms of testicular tumors which I took to originate from the epithelium in the seminiferous tubules or from the anlage of these tubules:

1. Seminoma (without production of chorionic hormone).
2. Mixed epithelioma (as a rule with production of chorionic hormone).
3. Small-cystic mixed tumor.

This classification has since proved serviceable in practice. The greater part of the testicular tumors may readily be classified as belonging to one of these three categories. The seminoma is a tumor of quite uniform structure, whereas the mixed epithelioma often is very variegated — as, for instance, it not infrequently contains seminomatous, adenocarcinomatous and papilliferous areas, sometimes with transitions to areas resembling chorionic epithelioma. The small-cystic mixed tumors will be left out of this discussion at present, as their histogenesis is difficult to assume and it may be that some of them are of embryonal origin, while others perhaps develop from the epithelium of the seminiferous tubules, like the two preceding groups.

Dr. Hamburger has demonstrated that the gonadotrophic hormone is formed in the tumors themselves or in their metastases. Most of the patients on whom these hormonal analyses were carried out had metastases. It has now been found that most of the tumors belonging to the group of mixed epithelioma produce chorionic hormone even though we did not succeed in demonstrating any chorionepithelioma-like areas in the tumor. In spite of this lack of histological demonstration, then, the tumor or its metastases nevertheless contain cells with the same secretory function as that of the trophoblasts. Hence I do not find it justifiable — as has been done by many investigators, including Dr. Teilum — to set up a particular form of tumor called »chorionepithelioma testis» because it is possible, chiefly by chance, morphologically to demonstrate the presence of chorionepithelioma-like areas. I find it preferable to apply a common designation collectively to the entire group of tumors with varying degrees of differentiation, showing production of chorionic hormone and proving biologically to be very malignant, and yet we may very well try to differentiate between various types of such tumors, according to the histological structure, etc. It is this group of tumors I have designated as »mixed epithelioma», laying the main stress on the fact that they are epithelial tumors varying in structure; and this designation is readily distinguished from the concept »mixed tumor», in which there is an admixture of tissue from one or both of the other germ-layers. The testicular chorionepithelioma (and the primary chorionepithelioma of the ovary) cannot be separated from this group as an independent tumor. As far as that goes in its structure it differs markedly from the chorionepithelioma arising from a hydatidiform mole in the placenta, as it is never a clear-cut chorionepithelioma, but is always encountered as chorionepithelioma-like islands in tumors of the mixed epithelioma group or in their metastases.

Ahlström further calls attention to the fact that the testicular chorionepithelioma belongs to this group also on another account. For, in contrast to the chorionepithelioma proper, this tumor shows a pronounced tendency to metastasis by way of the lymphatics — just like the seminomas and the mixed epitheliomas. Hence, I think that »chorionepithelioma testis» cannot be set up as a special form of tumor. Differentiation of trophoblasts and production of chorionic hormone are common to nearly all the tumors of the mixed epithelioma group.

Also the designation »seminoma» which Dr. Hamburger introduced rather against my wishes, is misleading. Morphologically, as far

as I have had an opportunity to examine this tumor, the »pseudoseminoma« is built like a clear-cut seminoma, and it has practically been impossible here to demonstrate such structures as encountered in mixed epithelioma. Nevertheless, this tumor produces chorionic hormone. Actually the pseudoseminoma may hardly be anything but a mixed epithelioma that has not been recognized properly. In this connection I may mention that in a case of this kind we were able on autopsy to find chorioneplitheliomatous areas in a metastasis to the liver. This observation is quite in keeping with the findings reported by Oberndorfer long before hormonal analyses were known, in a case of »seminoma with metastases of chorioneplithelioma«.

So the two groups, seminoma and mixed epithelioma may very well be differentiated even though the relationship is very close, and I think that the grouping outlined here is sufficient for ordinary practical use.

As is well known, the anlage is the same for the two kinds of gonads, the ovary and the testis. Later, under normal conditions, the medullary cords of the ovary disappear and the Pflüger cords develop with differentiation of the follicles. It is only reasonable to assume that remnants of the medullary cord may give rise to tumors in the ovary that are homologous with the testicular tumors. Ovariseminomas have long been known, and I think I have been able to show that the so-called »primary chorioneplithelioma« of the ovary is a mixed epithelioma (Congress of Pathologists, 1938). Now Dr. Teilum brings to this view the significant support implied in the findings that a certain group of ovarian tumors which Schiller, no doubt erroneously has interpreted as mesonephromas, are built quite like certain tumors with glomerulus-like areas that may be seen in the testis. But tumors of this kind belong just to the group of mixed epithelioma, and hence the demonstration of these tumors by Dr. Teilum means an additional confirmation of my view that this entire group as well as the seminomas may occur in the ovary, undoubtedly originating from remnants of the medullary cords. I therefore find it misleading to set up these tumors as a special group; I think they are interpreted more correctly as a subdivision of the »mixed epithelioma« group. As in the case of testicular tumors I find it preferable to divide these ovarian tumors into the following groups.

1. Seminoma.
2. Mixed epithelioma.
3. Small-cystic mixed tumor.

This grouping is clear and practical and ought to be adopted also for tumors arising from the medullary cords in the ovary. The last group has not been mentioned by Dr. Teilum at all. It would be of interest to learn whether Dr. Teilum in his investigations has met with instances of small-cystic mixed tumor.

It now remains to show whether these ovarian tumors behave like the testicular tumors as far as hormonal excretion is concerned. This seems most likely but it will be rather difficult to demonstrate because of the greater infrequency of these ovarian tumors.

As to the arrhenoblastomas and luteomas, these tumors constitute particular groups that fall outside the scope of the subject we have discussed here — as has also been pointed out by Dr. Teilum.

Gunnar Teilum: Referring to the findings and data I have already presented, I wish to say to Dr. Bang that it has not been practicable further to differentiate his group »epithelioma mixtum syncytiomatodes testis« as

no morphological delimitation of the various forms of tumors, which I have classified as intermediate — in conformity with their demonstrated homology with ovarian tumors — has been carried out previously as far as the testis is concerned. The misinterpreted, but morphologically well-defined, »mesonephroma of the ovary« set up by Schiller, without morphologically demonstrable combination with chorionepithelioma in a number of cases, show, however, that such a morphological definition is not only practicable but also required if we are going to make further advance in the morphological differentiation of the dysontogenetic ovarian tumors. Naturally, the same conditions will apply to the testicular tumors. In this field of work, which hitherto has been accessible but with difficulty, and to some extent had to be hypothetical, I think, that just the homologies here demonstrated have afforded us the means of — or the key to — such a joint classification of these ovarian and testicular tumors, which is the only rational grouping. As to the ovarian tumors, this classification also affords a better differentiation from other dysontogenetic tumors — as, for instance, the granulosa-cell group.

Transitional forms between the three forms of the seminoma group are seen not infrequently. In several instances of the intermediate form and in a few of the seminoma (dysgerminoma) type (pseudoseminoma) the presence of chorionepitheliomatous proliferations was revealed first by the hormonal analysis. On the other hand, I have seen several instances of the »intermediate type« without excretion of chorionic hormones, and in such cases the most reliable classification will be made morphologically.

Also in the case of the small-cystic testicular teratoma I have been able to demonstrate a morphological congruity with certain (cystic and teratoid) forms of Schiller's »ovarian mesonephroma«. Here, I think, the decisive point is the occurrence of totipotent cell material and its degrees and forms of differentiation in the individual case, quite corresponding to the other forms of differentiation in the seminoma group.

Erik Andreassen: *Significance of the Various Lymphoid Organs to the Lymphocyte Production in the Albino Rat.**)

Labelled phosphate is administered to adult rats by subcutaneous injection. After experimental periods of 2 and 42 hours the desoxyribose nuclei acid present in the various lymphoid organs is extracted. By comparison of the activity of 1 mg. nuclei acid P with the activity of 1 mg. plasma phosphate P, data on the rate of the desoxyribose nuclei acid formation are obtained.

A large nuclei acid turnover is found in the lymphoid organs. The most rapid renewal of nuclei acid takes place in the thymus, amounting to 2—5 times the rate of renewal found in lymph nodes, spleen and Peyer's patches.

Various considerations make it likely that the nuclei acid turnover affords an estimate of the lymphocyte production in the thymus to be the most important lymphocytopoietic organ. The lively lymphocyte output in the lymphoid organs does not support the assumption of a circulation.

J. Engelbreth-Holm expressed his appreciation of this most interesting paper, so full of suggestions, but felt the want of a discussion of

*) To be published in extenso in Acta path. et microbiol. Scandinav. Suppl. LIV, 1944.

the results here presented in comparison to Sjövall's investigations, in which experiments with bleeding made it probable that a lymphocytic circulation takes place.

The further investigations by Dr. Andreasen in this field are awaited with considerable interest.

J. Clemmesen would like to be informed whether it were possible to exclude a radioactive influence from the substance employed on the cell divisions of the lymphatic tissue.

Tage Kemp wanted, in connection with this interesting paper, to call attention to the fact that the pituitary growth hormone has a distinct, though transitory, stimulating effect on the thymus. As demonstrated by Fonns-Bech, numerous mitotic figures are seen in the thymus in dwarf-mice which are given colchicin and growth hormone simultaneously.

Mogens Andreasen: Two cases of right-sided aortic arch observed on autopsy.

(No abstract received.)

Discussion:

Niels Fiil demonstrated an instance of right-sided aortic arch in a child, 13 months old, who since birth had had numerous attacks, lasting from 30 sec. to 1 min., with stridor and cyanosis, and often with stridorous and laborious breathing between the attacks. These phenomena were aggravated markedly during the frequent »colds« to which the child was susceptible. During such an attack the child was admitted to the Epidemic Hospital for diphtheria, wherefrom the patient was transferred to the Ear Dep. of the Copenhagen County Hospital. Here the patient had several attacks like the above-mentioned, with rise in temperature and bronchitis.

Roentgenography revealed an impression on the right side of the trachea, just above the bifurcation, so that the trachea was constricted markedly. Tomography showed here a smooth round shadow, about 2 cm. in diameter, which was taken to be an enlarged lymph node; further, there was an increase in the retro-oesophageal space at the same level. Tracheoscopy showed a roundish intumescence, covered by mucous membrane (microscopy: sub-chronic tracheitis). After the tracheoscopy there was an exacerbation of the bronchitis, with a marked rise in temperature and very troublesome breathing; and the child died.

Autopsy showed a child of normal development and fair nutrition. The mucous membrane of the larynx and trachea was swollen, red, covered by mucus and pus; no stricture, impression or dislocation was seen. No tumor was found in the mediastinum. The thymus appeared normal. Pleurae normal. The lower lobe of the right lung was atelectatic. Otherwise the lungs were congested, without manifest pneumonia. The pericardium was normal. The heart normal as to position; it measured 4×4.5 cm. It showed hypertrophy of the left side (right ventricle 3 mm., left 9 mm.). No defect in the septum. Foramen ovale closed. Ductus Botalli obliterated. The pulmonary artery was normal. The aorta took its origin from the usual place, but instead of passing upwards to the left, it ran upwards to the right, passing over the right bronchus and past the trachea, immediately above the departure of the right bronchus anteroposteriorly, turning then to the

left, behind the oesophagus, passing the midline, and turning into a descending aorta of normal location. Thus the aorta embraced the trachea and oesophagus from the right.

Besides the aortic anomaly described, the autopsy revealed no abnormality.

J. Engelbreth-Holm: Three Cases of Myeloma with Transition to Reticulosarcomatosis.

(No abstract received.)

The paper will be published in extenso in *Acta pathologica et microbiologica Scandinavica*, Supplement LIV, 1944.

No discussion.

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ACTA PATHOLOGICA ET MICROBIOLOGICA SCANDINAVICA

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In ACTA PATHOLOGICA ET MICROBIOLOGICA SCANDINAVICA only articles written by Scandinavian authors are published; they are issued in English, French or German, according to the author's desire.

Subscribers are requested to apply to Ejnar Munksgaard, Publisher, Copenhagen, Nørregade 6. One volume (generally 4 numbers, ca. 6—700 pages) is published every year with numerous supplements. Each volume costs 35 Danish crowns.

Dans ACTA PATHOLOGICA ET MICROBIOLOGICA SCANDINAVICA ne sont publiés que des articles écrits par auteurs scandinaves; selon leur désir, ils seront publiés en français, anglais ou allemand.

Pour les abonnements on est prié de s'adresser au éditeur, M. Ejnar Munksgaard, Copenhague, Nørregade 6. Prix par volume Cr. Dan. 35.—.

In ACTA PATHOLOGICA ET MICROBIOLOGICA SCANDINAVICA werden nur Artikel von skandinavischen Verfassern veröffentlicht; den Wünschen der Verfasser gemäss erscheinen sie in deutscher, englischer oder französischer Sprache. Zu beziehen von der Verlagsbuchhandlung Ejnar Munksgaard, Kopenhagen, Nørregade 6. Preis pro Band 35 dänische Kronen.

I ACTA PATHOLOGICA ET MICROBIOLOGICA SCANDINAVICA optages som Regel ikke Afhandlinger større end 2 Ark (32 Sider). Manuskripter indsendes maskinskrevne, oversatte til Engelsk, Fransk eller Tysk, til en af de respektive Landes Redaktører.

ACTA PATHOLOGICA ET MICROBIOLOGICA
SCANDINAVICA
VOL. XXI

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VOL. XXI

1944.

EINAR MUNKSGAARD · KØBENHAVN

MCMXLIV

Printed in Denmark.

H. P. Hansens Bogtr., Kbhvn.

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EXPERIMENTELLE BEITRÄGE ZUR OLIGODYNAMISCHEN WIRKUNG MIT BESONDERER RÜCKSICHT AUF DIE WASSERREINIGUNG: II.

DIE KEIMVERGIFTUNG DURCH ALUMINIUM- UND KIESELSÄURE-
VERBINDUNGEN UND DEREN KOMBINATION MIT KUPFER U. A.,
UND DIE WIRKUNG DER METALLHYDROXYDE

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(Eingegangen bei der Redaktion am 14. Juni 1944.)

Dem Aluminium wird in der Desinfektionslehre nur eine sehr begrenzte keimtötende Wirkung zugeschrieben. Aluminium- wie Eisenhydroxyd sind schwache Basen, die Salze daher in wässriger Lösung hydrolytisch gespalten und reagieren sauer. Entsprechend ihrer Dreiwertigkeit haben die Aluminium- wie die Eisen- und Chromsalze starkes Fällungsvermögen für Kolloide und schrumpfende Wirkung auf Gallerten, heben daher die Plasmolyse pflanzlicher Zellen auf und setzen durch Erstarrung des Plasmas dessen Aufnahmevermögen für andere Stoffe herab. Die keimtötende Wirkung der Aluminiumsalze gilt als unerheblich und beruht vorwiegend auf der abgespaltenen Säure, etwas grösser ist die entwicklungshemmende.

Diese Weyls Handbuch entnommene Darstellung des Aluminium und seiner keimwidrigen Eigenschaften (1) ist in dieser Form nicht mehr aufrechtzuerhalten. Ist doch nach meinen Untersuchungen für das Aluminium eine erst in sehr hohen Verdünnungen hervortretende, keimtötende Wirkung charakteristisch, welche durch schwach alkalische Reaktion sowie durch minimale Mengen unter analogen Bedingungen wirksamer Stoffe — wie von Neutralsalzen, Schwermetallen, Halogenen, Kieselsäure u. a. m. — noch verstärkt wird. Eine solche Kombinationswirkung muss m. E. als wesentliches Merkmal oligodynamischer Wirkung angesehen werden (2).

Aluminiumsalze und deren Kombination mit anderen Stoffen.

Aluminiumchlorid wurde bei variierten pH-Werten in destilliertem Wasser gegen gelbe Staphylokokken und Paratyphus-B-Bazillen ge-

Versuch 87.

Bestimmung der Wirkungskurve des Aluminiumchlorids in destilliertem Wasser und in Phosphatgepufferten Lösungen verschiedener pH gegen a) *Staphylococcus aureus*, b) *Bac. typhi* mur. zu je 1/50 Öse. Aussaat nach 48 Stunden: Pufferung entspr. m/300.

AlCl ₃ :	$\frac{n}{100}$	$\frac{n}{1000}$	$\frac{n}{10000}$	$\frac{n}{100000}$	$\frac{n}{\text{Mill.}}$	$\frac{n}{10 \text{ Mill.}}$	$\frac{n}{100 \text{ Mill.}}$	aq.
a) aq.	2	1	±	3	4	4	—	4
5.9	—	—	3	4	—	4	2	4
6.6	—	—	4	4	4	4	4	4
7.3	—	4	4	4	4	4	4	4
8.0	—	4	4	4	2	3	—	—
b) ap.	±	±	1	4	4	4	±	4
5.9	—	±	1	2	3	3	3	3
6.6	—	4	4	4	4	4	4	4
7.3	±	4	4	4	4	2	4	4
8.0	±	4	4	4	2	4	4	4
Al: mg/l:	90.	9	0.9	0.09	0.009	0.0009	0.00009	.

Wegen der Technik und Methodik der Versuche und der Versuchsdarstellung verweise ich auf den Schluss der Einleitung meiner vorigen Arbeit in dieser Zeitschrift (XX, 92 ff., 1943). Die Zahlen, Striche und Kreuze in den Tabellen bedeuten: 4: etwa der Einsaat entsprechendes Wachstum, 3: etwa 2/4, 1: etwa 1/4 der Einsaat, ±: 1/8, ±: 1/16 und weniger, —: kein Wachstum (oder höchstens 20 oder weniger ausgewachsene Kolonien, bei 10000–20000 Keimen entspr. 1/1000 der Einsaat).

prüft (Versuch 87). In den höheren Konzentrationen des Salzes weist die saure Lösung, in den höchsten Verdünnungen dagegen die alkalische Lösung absolute Werte gegen *Staphylokokken*, relative gegen *Paratyphusbazillen* auf.

Durch Kochsalz werden (Versuch 89) diese absoluten Wirkungszonen gegen *Staphylokokken* u. zw. bei schwach alkalischer, nicht bei neutraler oder schwach saurer Reaktion, verstärkt und verbreitert u. zw. noch durch sehr geringe Kochsalzkonzentrationen — n/Millionstel und weniger, entspr. 0.058 mg NaCl/l. —, deren additive Wirkung mit z. B. Kupferchlorid ich eingehend beschrieben habe ((2) Versuch 74).

Analoges Verhalten, nur tiefere und breitere Wirkungszonen zeigt die Kombination Aluminiumchlorid und Kupferchlorid. Dabei wird die Kupferwirkung in starken und mittleren Konzentrationen durch

Versuch 89.

Grenzwertbestimmung einer Kombination von Kochstlz und Aluminiumchlorid in destilliertem Wasser und in Phosphatpufferlösung (m/300), bei pH-Grenzwerten, gegen Paratyphusbazillen. Einsaat 1/100 Öse, Aussaat etwa 1/1000 der Einsaat (entspr. etwa 10000 Keimen) nach 48 Stunden:

AlCl ₃ :	$\frac{n}{10000}$	$\frac{n}{100000}$	$\frac{n}{\text{Mill.}}$	$\frac{n}{10 \text{ Mill.}}$	$\frac{n}{100 \text{ Mill.}}$	aq.	mg/l
aq.pH 5.9	3	2	2	1	3	3	.
NaCl $\frac{n}{1000}$	4	4	3	3	4	3	» 58.5
» $\frac{n}{10000}$	4	3	1	2	2	2	» 5.85
» $\frac{n}{100000}$	4	4	3	4	3	3	» 0.585
» $\frac{n}{\text{Mill.}}$	3	3	3	2	1	2	» 0.0585
» $\frac{n}{10 \text{ Mill.}}$	4	4	3	3	2	2	» 0.0058
» $\frac{n}{100 \text{ Mill.}}$	3	3	2	2	2	2	» 0.0006
aq.pH 8.0	—	±	±	—	2	1	.
NaCl $\frac{n}{1000}$	4	3	±	±	1	1	» (wie oben)
» $\frac{n}{10000}$	—	2	2	—	—	2	»
» $\frac{n}{100000}$	4	2	±	±	1	2	»
» $\frac{n}{\text{Mill.}}$	—	2	1	±	—	2	»
» $\frac{n}{10 \text{ Mill.}}$	4	2	1	1	±	2	»
» $\frac{n}{100 \text{ Mill.}}$	4	2	1	1	±	1	»
Al:mg/l:	0.9	0.09	0.009	0.0009	0.00009	.	.

entsprechende Werte des Aluminiumsalzes sehr gehemmt, und tritt die additive Wirkung beider Metalle erst bei Verdünnungen zwischen n/Millionstel und n/10 Millionstel und darüber zutage. Diese Abtötungszonen kehren mit grosser Regelmässigkeit und an ziemlich gleicher Stelle der Reihen gegen Staphylokokken und Paratyphusbazillen wieder und entsprechen annähernd den oligodynamischen Phasen der

Versuch 104.

Aktionskurve des Aluminium und des Kupfers in destilliertem Wasser. Ansatz: Al-Verdünnungen zu 1 cm³, dazu Einsaat von je 1/100 Öse Paratyphus bazillen in je 0.8 cm³ nach Zeit und, nach halbstündiger Einwirkung des Al, Kupfer zusatz in destilliertem Wasser nach Zeit. Aussaat nach:

4 Stunden:

Al Cl ₃ :	$\frac{n}{10000}$	$\frac{n}{100000}$	$\frac{n}{\text{Mill.}}$	$\frac{n}{10 \text{ Mill.}}$	$\frac{n}{100 \text{ Mill.}}$	aq.	mg/1 Cu
CuCl ₂ $\frac{n}{10000}$	—	—	—	—	—	—	3.2
" $\frac{n}{100000}$	±	±	±	—	—	±	0.32
" $\frac{n}{\text{Mill.}}$	±	1	1	1	2	2	0.032
" $\frac{n}{10 \text{ Mill.}}$	±	1	2	2	±	3	0.0032
" $\frac{n}{100 \text{ Mill.}}$	±	1	1	2	3	3	0.0032
aq.dest.	—	±	4	2	4	4	.

24 Stunden:

CuCl ₂ $\frac{n}{10000}$	—	—	—	—	—	—	"
" $\frac{n}{100000}$	—	—	—	—	—	—	
" $\frac{n}{\text{Mill.}}$	—	±	—	±	±	±	
" $\frac{n}{10 \text{ Mill.}}$	—	±	±	±	±	±	
" $\frac{n}{100 \text{ Mill.}}$	—	±	±	±	1	2	
aq.dest.	—	±	±	±	2	3	
Al:mg/l:	0.9	0.09	0.009	0.0009	0.00009	.	

Komponenten. Deutlich treten bei der Kombination der höheren und höchsten Verdünnungen die zweiten Wirkungszonen hervor (Versuch 104).

Tritt die eigenartige Wirkungsweise des Aluminium schon in diesen Versuchen in destilliertem Wasser klar hervor, wobei die breiten Hemmungszonen zwischen den wirksamen höheren Konzentrationen bzw. höchsten Verdünnungen imponieren, in der Kombination mit

Versuch 92.

Lovöorchwasser im Grenzwertermittlungsversuch mit CuCl_2 und AlCl_3 gegen
1/50 Öse Paratyphus-B-Bazillen. Aussaat nach 24 Stunden:

AlCl_3 :	$\frac{n}{1000}$	$\frac{n}{10000}$	$\frac{n}{100000}$	$\frac{n}{\text{Mill.}}$	$\frac{n}{10 \text{ Mill.}}$	$\frac{n}{100 \text{ Mill.}}$	nq.	mg/1 Cu
CuCl_2 :								
$\frac{n}{1000}$	—	—	—	—	—	—	—	32.
$\frac{n}{10000}$	—	—	—	—	—	—	—	3.2
$\frac{n}{100000}$	—	—	—	—	—	—	—	0.32
$\frac{n}{\text{Mill.}}$	—	—	—	\pm	\pm	\pm	—	0.032
$\frac{n}{10 \text{ Mill.}}$	—	—	\pm	4	4	4	\pm	0.0032
$\frac{n}{100 \text{ Mill.}}$	—	—	—	\pm	4	4	\pm	0.00032
ap.dest.	—	—	—	3	4	4	4	.
CuCl_2 :								
$\frac{n}{1000}$	—	—	—	—	—	—	—	wie oben!
$\frac{n}{10000}$	\pm	—	—	—	—	—	—	
$\frac{n}{100000}$	2	\pm	—	—	—	—	—	
$\frac{n}{\text{Mill.}}$	4	4	2	2	2	1	\pm	
$\frac{n}{10 \text{ Mill.}}$	4	4	4	4	4	4	4	
$\frac{n}{100 \text{ Mill.}}$	4	4	4	3	3	3	4	
Lovöwasser pH: 7.3	4	4	4	4	4	4	4	(Einsaat rund 17 Mill. Keime.)
Al:mg/l:	9.	0.9	0.09	0.009	0.0009	0.00009	.	

Ansatz: Kupferverdünnungen, dazu Aluminiumverdünnungen in je 0.5 cc der
beiden Wässer, zuletzt nach Zeit Einsaat in 1.0 cc.

Kupfer ausserdem die starke Hemmung dieses Metalls weit über des-
sen Selbsthemmung hinaus, so erscheint das hier gezeichnete Verhal-
ten des Aluminium auch anschaulich in Versuchen unter technischen

Bedingungen. Hemmung wie Förderung der keimvergiftenden Wirkung anderer Substanzen durch Aluminium lassen sich auch in natürlichen Wässern von Wasserwerken und Badeanstalten nachweisen und geben den hier mitgeteilten Beobachtungen eine bisher nicht beachtete, praktische Bedeutung.

So erscheint die eben beschriebene Kupferhemmung durch Aluminium in destilliertem Wasser stets auch in natürlichen Wässern. Dadurch wird eine durchgreifende Desinfektion zeitlich u. U. ausserordentlich verzögert, besonders bei auch nur schwach saurer Reaktion (pH 6.5) (Versuch 92). Dagegen tritt bei auch nur schwach alkalischer Reaktion — etwa unterhalb eines Grenzwertes von $n/100000$ entspr. 0.09 mg Al/l — eine deutliche, kräftige Verstärkung der Kupferwirkung hervor. Dabei ist im Auge zu behalten, dass Aluminium auch allein — etwa von der Konzentration $n/100000$ an — eine starke Desinfektionswirkung entfaltet, welche gegebenenfalls durch Kochen des untersuchten Wassers noch bedeutend gesteigert wird. Es liegt nahe, solche Aluminiumwirkung unter technischen Verhältnissen als kombinierte Aluminium-Kupferwirkung aufzufassen, wobei das Kupfer von Vorrichtungen im Leitungs- und Filtersystem stammen dürfte (Versuch 106). Darüber gibt es ältere amerikanische Mitteilungen, nach welchen z. B. den Kupfernieten der Filter wesentliche keimtötende Wirkung auf Typhusbazillen, weniger auf Coli beigelegt wird (8).

In kombinierten Versuchen mit Jod oder Chlor, sowie Kupfer- und Aluminiumsalzen tritt — neben der früher von mir eingehend beschriebenen Halogen-Kupferhemmung (2, 7) — auch eine deutliche, zusätzliche Hemmung beider Komponenten durch Aluminium auf. Dies erscheint auch dann nicht unbeachtlich, wenn nach längerer Dauer die Kupferwirkung sich allmählich durchsetzt; und wenn in alkalischem Milieu (noch nicht chloriertes Reinwasser) die Kombination Halogen + NaCl (Neutralsalze) + CuCl_2 + AlCl_3 sich oligodynamisch als ausserordentlich wirksam erweist.

Die hier mehrfach besprochene Verstärkung der Kupferwirkung durch kleinste Dosen Aluminium tritt bemerkenswerterweise nur auf, wenn zuerst das Aluminiumsalz, darauf das Kupfersalz; umgekehrt Hemmung des Kupfereffektes, wenn zuerst das Kupfer- und darauf das Aluminiumsalz den Testkeimen zugesetzt wird. Diese Hemmung ist offenbar darauf zurückzuführen, dass das Kupfer in seiner Verteilung über die Zellen durch das nachfolgende Aluminium in eine stabilere, mehr oder weniger unlösliche Verbindung übergeführt wird; während kleine Mengen Aluminium ausserordentlich schnell an empfindliche Teile der Zellen gebunden werden, ohne eine additive Wirkung des Kupfers zu beeinträchtigen. Es liess sich also ein Einfluss der Reihenfolge der Zusätze auf den Entkeimungsprozess feststellen, der von ausschlaggebender Bedeutung für den Entkeimungseffekt ist, was bei der praktischen Desinfektion ebenso wie bei der Desinfek-

Aktionskurve des Al und Cu in Klaraseewasser (Ansatz: AlCl_3 -Verdünnungen zu 1 cm^3 , dazu Kulturaufschwemmung zu je $1/100$ Öse Paratyphusbazillen in 0.8 cm^3 Wasser nach Zeit, nach 18 Minuten Zusatz von CuCl_2 in 0.2 cm^3 nach Zeit). Aussaat nach a) 4 und b) 24 Stunden:

Gleichzeitig, aber ohne fallende Al-zusätze, mit Al-sulfat gefälltes und geklärtes Wasser sowie Schnellfiltrat der Reinigungsanlage des Sportpalastes in Stockholm.

AlCl_3 :	$\frac{n}{10000}$	$\frac{n}{100000}$	$\frac{n}{\text{Mill.}}$	$\frac{n}{10 \text{ Mill.}}$	$\frac{n}{100 \text{ Mill.}}$	aq.	mg/1 Cü
a)							
CuCl_2 : $\frac{n}{10000}$	—	—	—	—	—	—	3.2
» $\frac{n}{100000}$	\pm	—	\pm	—	—	—	0.32
» $\frac{n}{\text{Mill.}}$	4	3	4	4	4	4	0.032
» $\frac{n}{10 \text{ Mill.}}$	4	4	3	4	4	4	0.0032
» $\frac{n}{100 \text{ Mill.}}$	4	4	4	4	4	4	0.00032
Klaraseewasser pH: 7.2	4	4	4	4	4	4	.
desgl. gefällt pH: 6.5 CuCl_2 :	—	—	4	4	4	4	mg/1 Al (0.09
» filtriert u. gemischt, pH: 7.2	—	—	\pm	4	4	\pm	» } *)
» »(gekocht)	—	—	\pm	1	2	\pm	» } **)
CuCl_3 : $\frac{n}{10000}$	—	—	—	—	—	—	wie oben!
» $\frac{n}{100000}$	—	—	—	—	—	—	
» $\frac{n}{\text{Mill.}}$	3	3	4	1	4	2	
» $\frac{n}{10 \text{ Mill.}}$	4	4	4	2	4	4	
» $\frac{n}{100 \text{ Mill.}}$	4	4	4	4	4	4	
Klaraseewasser	4	4	4	4	4	4	4
mg/1 Al:	0.9	0.09	0.009	0.0009	0.00009	.	
desgl. gefällt pH 6.5 CuCl_2 :	—	—	2	4	4	4	wie oben!
» filtr. u. gemischt, 7.2	—	—	—	3	4	—	» } *)
» »(gekocht)	—	—	—	—	—	—	» } **)

Schon Rohwasser mit einem Keimgehalte von rund 10 Millionen Paratyphusbazillen im cm^3 wird von Kupferchlorid $n/100000$ — bei ausgesprochener Hemmung durch Al-sulfat, in vier Stunden fast vollkommen entkeimt, gefälltes und filtriertes bei Al-gehalt von $n/100000$ vollständig; das letztere nach Kochen weitaus am stärksten u. zw. auch ohne Kupferzusatz. *) Al-Cu-Wirkung der Kontrollen (durch Cu^{++} von den Filterdüsen?). **) Al-Cu-Wirkung der Kontrollen verstärkt durch von Glase beim Kochen abgelöste Stoffe.

Versuch 96.

Grenzwertbestimmung einer Kombination von AlCl_3 und CuCl_2 gegen Staphylokokken in Lovöwasser. Einsaat 1/100 Öse, Aussaat nach 24 Stunden. Versuchsanordnung abweichend von der üblichen in der Reihenfolge der Zusätze: a) Aussaat-Aluminiumsalz-Kupfersalz, b) Aussaat, Kupfersalz, Aluminiumsalz im Abstände von etwa zehn Minuten; Einsaatkontrolle etwa 10 000(000) Keime.

AlCl_3 :	$\frac{n}{100}$	$\frac{n}{1000}$	$\frac{n}{10000}$	$\frac{n}{100000}$	$\frac{n}{\text{Mill.}}$	$\frac{n}{10 \text{ Mill.}}$	$\frac{n}{100 \text{ Mill.}}$	aq.	mg/1 Cu
a)									
CuCl_2 $\frac{n}{100}$	\pm	\pm	—	—	—	—	—	—	320.
„ $\frac{n}{1000}$	\pm	\pm	—	—	—	—	—	—	32.
„ $\frac{n}{10000}$	\pm	\pm	—	—	—	—	—	—	3.2
„ $\frac{n}{100000}$	\pm	\pm	—	—	—	—	—	—	0.32
„ $\frac{n}{\text{Mill.}}$	\pm	—	1	—	—	—	—	\pm	0.032
„ $\frac{n}{10 \text{ Mill.}}$	\pm	\pm	1	\pm	\pm	\pm	\pm	1	0.0032
„ $\frac{n}{100 \text{ Mill.}}$	\pm	—	4	3	2	2	2	3	0.0003
aq.	\pm	\pm	4	3	2	2	2	2	.
b)									
CuCl_2 $\frac{n}{100}$	—	—	—	—	—	—	—	—	320.
„ $\frac{n}{1000}$	\pm	\pm	\pm	\pm	\pm	\pm	\pm	—	32.
„ $\frac{n}{10000}$	—	—	—	—	—	—	—	—	3.2
„ $\frac{n}{100000}$	—	—	—	—	—	—	—	—	0.32
„ $\frac{n}{\text{Mill.}}$	1	1	1	\pm	\pm	\pm	1	\pm	0.032
„ $\frac{n}{10 \text{ Mill.}}$	2	2	2	2	2	2	2	1	0.0032
„ $\frac{n}{100 \text{ Mill.}}$	3	3	2	3	3	3	3	3	0.0003
aq.	\pm	\pm	4	3	2	2	2	2	.
mg/1 Al:	90.	9.	0.9	0.09	0.009	0.0009	0.00009	.	.

Abhängigkeit der Wirkung von der Reihenfolge der Zusätze, entsprechend den Verhältnissen in kolloidalem Milieu.

tionsmittelprüfung zu berücksichtigen sein dürfte. Ich komme hierauf noch mehrfach zurück (Versuch 96).

In solchen Versuchen tritt überdies, wie schon oben erwähnt, neben der Aluminium-Kupferwirkung in ihrem gehemmten bzw. ihrem

additiv wirksamen Bereiche, die zusätzliche Wirkung von Alkali, besonders aber einer Alkali- und Kieselsäureabspaltung von keramischen Werkstoffen, Baustoffen, Filtern u. a. m., schliesslich auch vom Glase beim Kochen von Wasserproben in Erscheinung. Wegen der Grundlagen hierfür verweise ich auf die erste Mitteilung dieser Reihe, in welcher ich auf die Lösung von Glas durch Wasser, Salze und Alkalien sowie die Bildung von Kupfersilikaten näher eingegangen bin ((2) Seite 88 ff. und 124). Ausser den genannten Stoffen ist schliesslich noch mit anderen Faktoren wie zusätzlichen Kupfermengen u. a. Stoffen aus dem Leitungssystem zu rechnen. Ich komme darauf nochmals zurück bei der Erörterung der zahlreichen Versuche mit gemessenen Mengen mehrerer Aluminium- und Kieselsäurepräparate sowie mit Kupfer, welche einen Zweifel an der Berechtigung der hier gemachten Annahmen nicht begründen.

Aluminiumhydroxyd.

Um bei der Prüfung der Aluminiumverbindungen von den starken anorganischen Säuren unabhängig zu sein, welche bei dem schwach basischen Charakter des Metalls ihre überwiegende Wirkung geltend machen müssen, habe ich das reine Aluminiumoxyd (Aluminium oxydatum hydricum Merck) in wässriger Verdünnung verwendet. In der Konzentration $n/25000$, entsprechend 10 mg des 7—8-prozentigen Präparates in 500 cm³ doppeldestillierten Wassers, dürfte sich alles Aluminiumoxyd in Lösung befunden haben.

Paratyphusbazillen werden von abnehmenden Konzentrationen dieser praktisch neutralen Lösung zwischen $n/\text{Millionstel}$ und $n/\text{Milliardstel}$ zunehmend angegriffen, besonders aber Staphylokokken, welche geradezu spezifisch beeinflusst werden. Bei der Kombination von Aluminiumhydroxyd und -chlorid zeigten sich in höheren Konzentrationen von $n/1000$ bis $n/100000$ sowie in höchsten Verdünnungen von $n/10$ Millionstel bis $n/\text{Milliardstel}$ relative bis absolute Wirkung, dazwischen im wesentlichen starke Hemmung (Versuch 110). Aluminiumoxyd und -chlorid wirken zweiphasig. Das Chlorion wirkt dabei anscheinend auf Al in ähnlicher Weise als hemmender Faktor wie auf Cu in meinen früheren Versuchen ((2) Versuch 74, dort auch Litteratur zur antagonistischen Wirkung des Chlors auf Kupfer).

Dieses Verhalten ist aber keineswegs eindeutig. Bald erscheint nämlich das Oxyd, bald das Chlorid in höchsten Verdünnungen wirksamer, infolgedessen im Kombinationsversuch bald das eine, bald das andere gehemmt. Welche Rolle dabei der Zeitfaktor, nämlich das Verweilens der Lösung im Versuchsglase und die Reihenfolge der Zusätze, weiterhin die Kulturmenge und die Nährbodenbeschaffenheit, schliesslich das Alter der Lösungen und etwa von den Aufbewahrungs-bzw. den Versuchsgläsern abgelöste Stoffe spielen, habe

Versuch 110.

Kombination von Aluminiumchlorid und Aluminiumoxyd. Al_2O_3 ist seiner Löslichkeit in Wasser entsprechend ($n/17300$) erst von $n/100000$ ab in Versuch gebracht; Einsaat je 1/50 Öse a) Paratyphus-Bac., b) gelbe Staphylococcen, Aussaat je 1/1000 der Einsaat n. 24 Stunden:

AlCl_3 :	$\frac{n}{100}$	$\frac{n}{10000}$	$\frac{n}{100000}$	$\frac{n}{\text{Mill.}}$	$\frac{n}{10 \text{ Mill.}}$	$\frac{n}{100 \text{ Mill.}}$	aq.	mg/1 Al
a)								
Al_2O_3 $\frac{n}{100000}$	—	\pm	\pm	1	1	4	4	0.09
Para- $\frac{n}{1000000}$	—	—	\pm	3	2	2	4	0.009
ty- $\frac{n}{10000000}$	—	—	—	1	\pm	3	4	0.0009
phus- $\frac{n}{100 \text{ Mill.}}$	—	—	\pm	2	3	1	4	0.00009
mur- $\frac{n}{\text{Milliard.}}$	—	\pm	\pm	2	2	3	3	0.000009
aq.	—	—	—	\pm	1	2	2	.
b)								
Al_2O_3 $\frac{n}{100000}$	—	\pm	4	3	3	2	4	wie oben!
Sta- $\frac{n}{\text{Mill.}}$	—	—	2	\pm	\pm	\pm	\pm	
phy- $\frac{n}{10 \text{ Mill.}}$	—	—	1	\pm	\pm	\pm	—	
lo- $\frac{n}{100 \text{ Mill.}}$	—	\pm	\pm	—	\pm	—	—	
coc- $\frac{n}{1000 \text{ Mill.}}$	—	—	—	—	—	—	—	
cus- $\frac{n}{1000 \text{ Mill.}}$	—	—	—	—	—	—	—	
aur. $\frac{n}{1000 \text{ Mill.}}$	—	—	—	—	—	—	—	
aq.	—	—	4	3	\pm	2	\pm	
mg/1 Al:	9.0	0.9	0.09	0.009	0.0009	0.00009	.	

ich bisher nicht ermitteln können. Da es sich hier um Gammawerte höchst wirksamer Körper handeln muss, welche chemisch nachzuweisen grossen Schwierigkeiten begegnet, so sind diese Fragen vorderhand methodisch nicht zu beantworten.

Auch hier hat die Reihenfolge der Zusätze u. U. ausschlaggebende Bedeutung für den Entkeimungserfolg. Die blosse Umkehrung z. B. der Reihenfolge zweier Zusätze, bei welcher das eine Mal die AlCl_3 -Kontrollen, das andere Mal die $\text{Al}(\text{OH})_3$ -Kontrollen etwa je zwölf Minuten länger als im anderen Ansatz in den Gläsern gestanden haben, bewirkt für das Chlorid ein umwälzendes Ergebnis. Steht es länger im Glase, so wirkt es verstärkt, während das Oxyd im analogen Falle keine stärkere Wirkung aufweist. Die Folge ist eine Wirkungsverstärkung im ersteren Falle über die reine Chloridwirkung hinaus.

Setzt man aber den Versuch so an, dass zuerst das Chlorid, dann die Einsaat und zuletzt das Oxyd zugefügt, im Parallelversuch da-

gegen zuerst das Oxyd, darauf die Einsaat und schliesslich das Chlorid eingebracht wird, so ist die Wirkung in jedem Betrachte die umgekehrte wie im vorigen Versuche. Es ist dabei zu beachten, dass im vorigen Versuche die wirkenden Stoffe einerseits mit dem Glase, andererseits unter einander in Verbindung treten, ehe sie auf die Keime einzuwirken haben, im zweiten Versuche aber der jeweils zuerst zugesetzte wirkende Körper zunächst mit dem Versuchsglase, dann mit den Testkeimen und erst zuletzt mit dem anderen wirkenden Körper in Verbindung tritt. Hier sind so verschiedene Möglichkeiten mehr oder weniger reversibler, komplexer Bindungen der wirksamen Komponenten möglich, dass die verschiedenen Versuchsergebnisse nicht zu überraschen brauchen. Bei jedem Entkeimungssystem handelt es sich um den Einfluss von Elektrolyten auf Suspensions- und Emulsionskolloide, durch den die Plasmahaut oder gewisse Innenkörper der Zelle oder der Kern selbst in Mitleidenschaft gezogen werden. Nach den grundlegenden Untersuchungen von Naegelis muss man sich die Behandlung von Keimsuspensionen mit verschiedenen Konzentrationen von Metallsalzen als Vergiftungen verschiedener Teile des Zellkörpers vorstellen. Treten nun Kombinationen von mehreren Stoffen in Aktion, so ist deren Wirkungsgrad ausser von den absoluten und relativen Mengen von der Reihenfolge abhängig, in welcher sie in Wirksamkeit treten. Von welcher Bedeutung für die Beurteilung von Desinfektionsabläufen solche wechsellvollen Ergebnissen aber sein müssen, welche Fehlerquellen in der blossen Versuchsanordnung verborgen sein, und welche Schwierigkeiten diese einer Aufklärung bieten können, ergibt sich aus dem Vorstehenden von selbst.

Aluminiumoxyd und Kupferoxyd.

Dem biochemischen Nachweise Spiros, dass der oligodynamischen Kupferwirkung eine Cupriionenwirkung zugrunde liege, habe ich direkte Versuche mit Cuprihydroxyd gegenübergestellt u. zw. in Kombination mit Aluminiumhydroxyd. Zur Kontrolle liefen entsprechende Reihen der Chloride nebenher. Der Doppelversuch wurde gegen Staphylokokken, Paratyphusbazillen und Subtilissporen geprüft ((8) Versuch 20). Durchgehend zeigen diese Versuche eine bedeutend stärkere Wirkung der Oxyde. Dabei erscheint die Wirkung des Aluminium der des Kupfers gegen Subtilissporen bedeutend überlegen. Die Kombination der beiden Oxyde weist überall additive Wirkung auf, während die Kombination der Chloride vergleichsweise starke, durchgehende Hemmung darbietet, besonders gegen Paratyphusbazillen und Subtilissporen. Die verwendete Cuprihydroxydlösung war in Ammoniak vorgenommen und entsprach bei $n/250$ $\text{Cu}(\text{OH})_2$ etwa $n/10$ NH_4OH . In den Versuchen wurden Verdünnungen von $n/100000$ bis

n/Milliardstel $\text{Cu}(\text{OH})_2$ bzw. n/4000 bis n/40 Millionstel NH_4OH verwendet.

Den Versuchen in destilliertem Wasser analog verliefen solche in Proben verschiedener Stadien des Wasserreinigungsprozesses von Mälarseewasser, in welchen die Wirkung von Kupferchlorid und Kupferhydroxyd auf Paratyphus-B-Bazillen verglichen wurde. Dabei zeigte sich die Überlegenheit des Hydroxyds über das Chlorid. In solchen Versuchen dürfte die Peptisierung des Kupferhydroxyds durch Ammoniak von wesentlicher Bedeutung für die Keimvergiftung sein. Auch hier ist, wie oben erwähnt, an additive Verstärkung des Kupfers durch zusätzliche Ionen aus dem Leitungs- und Filtersystem zu denken.

Kieselsäure.

Kieselsäure und kieselsaure Verbindungen haben durch die Verwendung von Natronwasserglas bei der Konservierung von Eiern und von Salzen der Siliciumfluorwasserstoffsäure in der technischen Desinfektion gegen Bakterien und Schimmelpilze Beachtung erfahren. Eine Reihe von Kieselsäurepraeparaten hat bei der Tuberkulose Anwendung gefunden. Im Zusammenhange mit der Pathologie der Silikose ist die Kieselsäure eingehend untersucht worden. Die Forschung ist noch im Flusse.

Nach Vorversuchen mit verschiedenen Lösungen von SiO_2 , SiO_2Na_2 und des Praeparates Siliquid, welche schon die charakteristische, zweiphasige Wirkung hoher Verdünnungen des Silicium aufwiesen, beschränkte ich mich weiterhin auf die Verwendung einer alkalischen Lösung von reinem SiO_2 , wie es für Silikoseversuche an Tieren verwendet wird. Die Lösung entsprach n/250 SiO_2 in n/100 NaOH und wirkte kräftig gegen die Testkeime, besonders gegen Staphylokokken.

Hatten schon die Vorversuche synergistische Effekte noch in ausserordentlich hohen Verdünnungen von Aluminiumoxyd und Kieselsäure nachweisen lassen, so bestätigte sich dieses in den folgenden Versuchen, welche einem Vergleiche der Wirkung der Kombinationen $\text{SiO}_2 + \text{Al}_2\text{O}_3$ und $\text{SiO}_2 + \text{AlCl}_3$ in verschiedenen Folgen galten. Die verwendeten Lösungen waren für Kieselsäureanhydrid die eben beschriebene Bereitung entsprechend n/250 SiO_2 in n/100 NaOH, für Al_2O_3 und AlCl_3 die oben bei den Versuchen 110 ff. verwendeten. Wie aus Versuch 113 hervorgeht, greift Kieselsäure in Verdünnungen zwischen n/100000 und Miliardstel Staphylokokken stark an. Analoges Verhalten zeigt Aluminium u. zw. besonders in der Oxydform. Der Alkaleszenz- oder Säuregrad der Lösungen fällt nicht mehr störend ins Gewicht. Dagegen dürfte die eingebrachte Kulturmenge von um so grösserer Bedeutung für die Versuchsergebnisse sein. Darauf habe ich schon anlässlich analoger Versuche mit Kupfer und Kochsalz

Versuch 113.

Kombination von Kieselsäureanhydrid ($n/250 \text{ SiO}_2$ in $n/100 \text{ NaOH}$) mit Aluminiumchlorid bzw. Aluminiumhydroxyd in reinem Wasser gegen Staphylokokken. Einsaat je $1/50$ Öse in 2 cm^3 Ansatz. Aussaat etwa je $1/500$ der Einsaat nach 24 Stunden:

SiO_2 :	$\frac{n}{100000}$	$\frac{n}{\text{Mill.}}$	$\frac{n}{10 \text{ Mill.}}$	$\frac{n}{100 \text{ Mill.}}$	$\frac{n}{\text{Milliard.}}$	aq.	mg/1 Al
AlCl_3 : $\frac{n}{100000}$	4	\pm	4	2	2	4	0.09
" $\frac{n}{\text{Mill.}}$	4	1	1	3	3	3	0.009
" $\frac{n}{10 \text{ Mill.}}$	2	\pm	4	4	4	3	0.0009
" $\frac{n}{100 \text{ Mill.}}$	3	\pm	2	4	4	3	0.00009
" $\frac{n}{\text{Milliard.}}$	2	\pm	1	\pm	\pm	3	0.000009
aq.	1	—	\pm	\pm	2	4	.
Al(OH)_3 $\frac{n}{100000}$	2	\pm	\pm	\pm	3	\pm	0.09
" $\frac{n}{\text{Mill.}}$	\pm	\pm	1	1	2	\pm	0.009
" $\frac{n}{10 \text{ Mill.}}$	\pm	—	—	\pm	\pm	\pm	0.0009
" $\frac{n}{100 \text{ Mill.}}$	—	—	—	—	—	—	0.00009
" $\frac{n}{\text{Milliard.}}$	—	—	—	—	—	—	0.000009
aq.	2	\pm	\pm	\pm	2	4	.
mg/1 Si:	0.07	0.007	0.0007	0.00007	0.000007	.	

Kieselsäure greift in höchsten Verdünnungen von $n/\text{Millionstel}$ bis $n/\text{Milliardstel}$ Paratyphusbazillen, besonders aber Staphylokokken oligodynamisch stark an. Analoges Verhalten, aber weit stärkere Wirksamkeit zeigt Aluminium u. zw. in der Oxydform, in welcher es hier weit stärker als das Chlorid wirkt. In den höheren Konzentrationen hemmen sich Al und Si, besonders in Gegenwart von Cl-Ionen.

hingewiesen ((2) Versuch 74). In den höheren Konzentrationen hemmen sich Kieselsäure und Aluminium, besonders in Gegenwart von Chlorionen. In den höchsten Verdünnungen aber, besonders in der Kombination $\text{SiO}_2 + \text{Al(OH)}_3$ nimmt die Wirkung sogar noch zu. Dies Verhalten konnte in einer ganzen Folge von analogen Versuchen gegen Staphylokokken und Paratyphusbazillen festgestellt werden, wobei auch auf den Einfluss wechselnder Reihenfolge geachtet wurde.

Hier erhebt sich die Frage, wie weit bei den enormen Verdünnungen, in welchen die maximale Wirkung von Kieselsäure und Alu-

minium eintritt, mit wesentlichen Verbesserungen chemisch-analytischer Distinktion der Versuchsbedingungen gerechnet werden kann. Solche dürften am ehesten in der Richtung einer Verwendung möglichst frischer Lösungen, möglichst einwandfreien Glases der verwendeten Mess- und Versuchsgeräte, u. U. in der Anwendung von Quarzgefäßen sowie reinstem Wasser, und schliesslich solcher Nährböden liegen, bei welchen nur mit wenigen Grundstoffen bekannter anorganischer und organischer Verbindungen gerechnet zu werden braucht.

Auch Kieselsäure habe ich, wie die Aluminiumverbindungen, mit Kupferchlorid und -hydroxyd kombiniert und vergleichend gegen Paratyphusbazillen geprüft. Alle Komponenten wirken zweiphasig, das Kupferoxyd bis hoch hinauf in die Region der Gammawerte (0.3—0.03). Beide Kupferverbindungen werden in der ersten und zweiten Phase durch Kieselsäure und entsprechend deren eigener Wirkungsphase additiv verstärkt. Dabei wirkt die Kombination mit Kupferhydroxyd im ganzen stärker als die mit Chlorid.

Schliesslich habe ich auch SiO_2 und $\text{Cu}(\text{OH})_2$ in verschiedener Reihenfolge untersucht und auch hier den Wirkungsunterschied feststellen können, welchem wir oben bei der Kombination $\text{AlCl}_3 + \text{CuCl}_2$ und seither noch mehrfach begegnet sind. Hier ist es die Folge $\text{SiO}_2 + \text{Cu}(\text{OH})_2$, welche starke Zunahme der Wirkung jenseits der Indifferenzzone aufweist und damit an die Wirkungssteigerung des Kupferhydroxyds in Verbindung mit Aluminiumoxyd im Vergleich mit den beiden Chloriden erinnert ((8) Versuch 120).

Erörterung.

Vergegenwärtigen wir uns noch einmal die Wirkungsweise der beiden hier näher geprüften Stoffe, so ist das charakteristische Merkmal beider darin zu erblicken, dass sie jenseits einer breiten Indifferenzzone ausserordentlich breite Wirkungszonen aufweisen. Gegen Staphylokokken wirkt dabei Aluminium zunehmend absolut noch in Konzentrationen von 0.9 bis 0.009 γ /l. Die Wirkung des Aluminium gegen Subtilissporen ist der des Kupferhydroxyds bei weitem überlegen. Kieselsäure erreicht die Werte der Aluminiumverbindungen nicht, wirkt aber u. U. noch in Konzentrationen von 7 bis 0.07 γ /l sehr stark keimvermindernd auf Staphylokokken und Paratyphusbazillen. Aluminium und Kiesel wirken unter den gewählten Bedingungen ähnlich wie hochmolekulare, organische Körper der Pyridin- und Chinolinreihe, welche in gepufferter, wässriger Lösung erst nach breiten Hemmungszonen zwischen 1000- bis millionfacher Verdünnung keimtötend in Wirksamkeit treten (3).

Neben diesem bakteriziden Eigenverhalten ist aber die Förderung oder Hemmung anderer Stoffe oder Kombinationen von solchen mit keimwidriger Wirkung durch Aluminium und Kiesel von theoretisch

schem wie praktischen Interesse. Bei der Wasserreinigung z. B., in deren Verlaufe die oligodynamische Zellvergiftung eine so bedeutende Rolle spielt (2), wurde Aluminium bisher nur als Fällungsmittel verwendet. Ich habe einige Anhaltspunkte geben können, welche die hemmende bzw. fördernde Wirkung des Aluminium z. B. auf die Halogene J und Cl, auf Neutralsalze wie NaCl, auf CuCl_2 , auf SiO_2 u. a. dartun, ohne angesichts der unmessbaren Verhältnisse bei diesen Prozessen bestimmte Grenzwerte fixieren zu können. Dies kann auch nicht wundernehmen bei so verwickelten Verhältnissen in kolloidalem Milieu und ist praktisch insofern von geringerem Belange, als Aluminium und Kiesel, ebenso wie die Neutralsalze der Alkali- und Erdalkalireihe, ferner gewisse Schwermetalle (Cu, Ag) und die Halogene, welche für die Oligodynamie der Wasserdesinfektion in Frage kommen, in schwach alkalischem Milieu (7.1 pH) sämtlich eine additive Verstärkung erfahren.

Mit diesem allgemeinen Wirkungsverhalten der beiden Stoffe erhebt sich aber als methodisches Problem die Frage der Brauchbarkeit des Glasversuches in seinen heutigen Formen für die Desinfektionsmittelprüfung in wässriger Phase. Bei der Spezifität des Aluminium z. B. gegen Staphylokokken und Subtilissporen müssen Spuren von Al, welche sich einem Nachweise entziehen, u. U. zu groben Täuschungen Anlass geben. Auch die Verstärkung, welche Spuren von $\text{Al}(\text{OH})_3$ z. B. für $\text{Cu}(\text{OH})_2$, oder Spuren von SiO_2 , die an sich kaum einer relativen Wirkung fähig sein mögen, für $\text{Al}(\text{OH})_3$ oder für $\text{Cu}(\text{OH})_2$ mit sich bringen, kann gegebenenfalls Wirkungen vortäuschen, welche einem gerade geprüften Stoffe gar nicht zukommen. Durch Kochen des verwendeten Wassers (Sterilisation) können diese Verhältnisse noch kompliziert, durch wechselnde Reihenfolge der Zusätze umgekehrt werden. Es besteht daher ein methodisches Interesse, diesen Glasfehler genauer zu bestimmen. Auf die Grundlagen hierfür habe ich in der vorigen Arbeit dieser Folge hingewiesen (2). In welcher Richtung methodisch ein Ausgleich der angedeuteten Fehlergrenzen zu suchen sein möchte, habe ich oben (Seite 827/828) erörtert.

Schliesslich ist noch die Bedeutung der Oxyde für den Vergiftungsvorgang bei der oligodynamischen Wirkung der Metalle zu erörtern. Schon aus von Naegelis Untersuchung (4) ging hervor, dass das Metall bei seiner Lösung in Wasser zunächst einem Oxydationsprozesse unterliegt. Spiro (5) konnte diese Erkenntnis dahin erweitern, dass die Geschwindigkeit dieses Oxydationsprozesses und mithin die Intensität des darauf gründenden Vergiftungsprozesses von der Gegenwart geeigneter Komplexbildner abhängt (NH_4 und Derivate) und nach Art einer Adsorptionskurve verläuft; dass ferner bei der oligodynamischen Wirkung des Kupfers Cupriionen wirksam sind. In der Desinfektionsmittelprüfung sind aber m. W. bisher Metalloxyde ebenso wenig untersucht als technisch verwendet worden. Die Kombination der Hydroxyde des Kupfers und des Aluminium weist nun eine starke Überlegenheit über die der Chloride auf und zwar auch in natür-

lichen Wässern. Diese Feststellung lenkt die Aufmerksamkeit auf praktisch-technische Versuche mit Kupfer und Aluminium bei der Wasserdesinfektion. Gesundheitliche Bedenken liegen hier nicht vor.

Eine technische Bemerkung ist noch hinsichtlich des Aluminiumsulfatzusatzes in Wasserwerken zu machen. Wegen der nicht geringen Möglichkeit einer Hemmung anderer bei der Wasserreinigung wirksamer Stoffe, insbesondere des Chlors, ist eine obere Grenze der Aluminiumkonzentration zweckmässigerweise nicht zu überschreiten und dürfte etwa bei einem Lösungsrest entsprechend $< n/100000$ liegen. Schon bei der Vorfällung ist darauf zu achten, den Aluminiumsulfatzusatz in möglichst engen Grenzen zu halten. Darüber hinaus aber ist in jedem Falle im Auge zu behalten, dass die Reaktion nach Abschluss der Wasserreinigung nicht auf der sauren Seite verharret. Nach mir bekannten Analysen von Trink- und Brauchwasser kommen in gereinigten Wässern Aluminiummengen vor, welche mit einer ausreichenden Entkeimung unvereinbar erscheinen.

Welche Rolle in diesem Zusammenhange Silicium spielen könnte, ist im wesentlichen erörtert worden. Neben der Beachtung aber, welche die Kieselsäure offenbar in der Oligodynamie verdienst, gewinnt noch eine andere Frage an Bedeutung, ob nämlich bei der menschlichen Silikose eine physikalische oder chemische Wirkung der mineralischen Silikate auf das Lungengewebe vorliege. Bei der Zellvergiftung im oligodynamischen Bereiche des Silicium wie des Aluminium und anderer Körper der organischen Reihe (Pyridine und Chinoline) handelt es sich offenbar um einen chemischen Angriff höchster Verdünnungen im Gegensatz zu konzentrierteren Lösungen, wie es dem ionalen Verhalten dieser Stoffe entspricht. Dabei ist der wirkungstergende Einfluss alkalischen Milieus wichtig, in welchem die Kieselsäure in Lösung geht. Mit Rücksicht auf die oben gestellte Frage ist es also nicht uninteressant zu wissen, dass Silicium eine zellschädigende Wirkung eigener Art auf vegetative Keime ausübt. Auch bei der Silikose kommt also wohl eine direkte Zellschädigung zustande.

Auch die Frage des Einflusses von Aluminium auf den silikotischen Prozess dürfte in den Wirkungskurven des Silicium und Aluminium an vegetativen und Dauer-Formen Anhaltspunkte finden. Soweit Bakterienvergiftung überhaupt Analogien für das zellpathologische Geschehen bei Metazoen bieten kann, ist angesichts der hier beschriebenen Wirkungsverhältnisse im Auge zu behalten, dass die höchsten Wirkungswerte und die breitesten Wirkungszonen im Bereiche von Gammawerten auftreten. Hier treten unter gewissen Bedingungen auch additive Wirkungen hervor, während die beiden Stoffe in höheren Konzentrationen an und für sich wie auch kombiniert oder gegen andere keimschädigende Stoffe mehr oder weniger Hemmung aufweisen.

Vergleicht man diese Konzentrations-Wirkungsbeziehungen mit den bei der Inhalation gegebenen Verhältnissen, so dürfte Kieselsäure

in den Schleimhäuten der Luftwege hinlänglich oligodynamisch löslich sein, ebenso auch metallisches Aluminium bzw. sein Oxyd in Lösung gehen, wobei wenigstens teilweise die entstehenden Polykieselsäuren durch Aluminate substituiert werden könnten. In Ansehung aber der minimalen Mengen dieser Stoffe, wie sie im Mechanismus der oligodynamischen Wirkung in Aktion treten, könnte auch eben-
sogut mit additiven Wirkungen gerechnet werden. Die bisherigen Erfahrungen über die eine Beeinflussung der Silikose durch Aluminium entsprechen eher der letzteren Alternative (6).

Zusammenfassung.

Das leicht lösliche Aluminiumchlorid wirkt in destilliertem Wasser mässig stark keimtötend, in schwach alkalischer Lösung dagegen erst in sehr hohen Verdünnungen zwischen n/Millionstel und n/Milliardstel u. zw. spezifisch gegen Staphylokokken, weniger stark gegen Paratyphusbazillen.

Noch in hohen Verdünnungen aber — um n/100000 entspr. 0.09 mg/l Al — wirken Aluminiumsalze hemmend auf andere oligodynamisch wirksame Stoffe wie Neutralsalze, Schwermetallsalze, Halogene u. a., sowohl im Modellversuch in destilliertem Wasser als auch unter technischen Bedingungen bei der Wasserdesinfektion in Wasserwerken und Schwimmbadanlagen. Bei alkalischer Reaktion treten in höheren Verdünnungen additive Effekte auf.

Von besonders starker Wirkung gegen Staphylokokken und Subtilissporen, weniger gegen Paratyphusbazillen, ist das reine, in Wasser nur in Spuren lösliche Aluminiumoxyd. Diese Wirkung auf vegetative Keime und Sporen tritt sowohl an sich wie auch besonders in Kombination mit anderen Giftstoffen z. B. mit Kupferchlorid und am stärksten mit Cuprihydroxyd in Erscheinung.

Bei diesen eigenartigen Wirkungen des Aluminium spielt die Reihenfolge der Zusätze eine für den Wirkungsgrad ausschlaggebende Rolle welche in bislang ungeklärten biochemischen Verhältnissen gründet.

Das Silicium verhält sich im ganzen ähnlich wie Al. Auch SiO_2 wirkt erst in den höchsten Verdünnungen $< \text{n/Millionstel}$ kräftig auf vegetative Keime ein, besonders auf Staphylokokken. Auch in Kombination mit Aluminium- und Kupfersalzen und -hydroxyden treten additive Wirkungen bei Gammawerten der einzelnen Stoffe, in höheren Konzentrationen breite Hemmungen zutage. Aluminium und Silicium zeigen hierin ein ähnliche Verhalten wie hochmolekulare Körper der Pyridin- und Chinolinreihe, welche in wässriger Lösung auch erst in sehr hohen Verdünnungen u. zw. in alkalischem Milieu zur Wirkung gelangen und unter Umständen noch durch Eiweisskörper eine Verstärkung erfahren.

Die Verstärkung der genannten Kupferverbindungen durch SiO_2 entspricht dessen zweiter Wirkungsphase, wobei auch wieder die Reihenfolge der Zusätze von wesentlicher Bedeutung für den Entkeimungseffekt ist. Dabei genügt schon die Umkehrung der Reihenfolge beim Zusatze der beiden Körper vor der Einsaat, um *ceteris paribus* stark differierende Wirkungskurven hervorzurufen.

Die Kombination der Hydroxyde des Al und Cu ist der der Chloride weit überlegen, auch in natürlichen Wässern.

Die Bedeutung der beschriebenen Befunde für die Methodik und Technik des Desinfektionsversuchs, insonderheit für die Theorie der Oligodynamie, für den Vitroversuch (Glasfehler) und schliesslich für einige praktische Fragen der Wasserreinigung werden erörtert.

Die Frage der Silikose und einer vorbeugenden Aluminiumwirkung wird gestreift.

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Abgeschlossen: Stockholm, den 21. November 1942.

Barnhusgatan 4, IV.

Gekürzt und ungeschrieben den 19. April 1944.

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PURIFICATION AND CONCENTRATION OF STREPTOCOCCAL FIBRINOLYSIN FROM BROTH CULTURES.

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(Received for publication June 14th, 1944).

Tillett and *Garner* found in 1933 that haemolytic streptococci produce an active substance able to dissolve fibrin clots. They named this substance streptococcal fibrinolysin.

The streptococcal fibrinolysin, which its discoverers took to be a proteolytic ferment, and the antibody formed in streptococcal infections have during recent years attracted great interest, special attention being focussed on the serological problems, i. e. the action of the anti-fibrinolysin in different streptococcal infections and their after effects, particularly rheumatic fever.

Already in 1934 *Tillett* and *Garner* tried to concentrate and purify the streptococcal fibrinolysin from broth cultures. By means of adsorption with aluminum hydroxide and elution with alkaline phosphate they succeeded in obtaining solutions of fibrinolysin which, in their lytic action, were about 16 times stronger than the original broth filtrate. It is impossible to assess the actual degree of purification obtained in this way, as the authors have not given the dry weights in their different preparations. *Tillett* and *Garner* have also shown that the fibrinolysin can be precipitated from broth filtrates with alcohol and acetone, and by salting out with ammonium sulphate. They have also shown that it is destroyed very quickly by trypsin and papain and must therefore in all probability be a protein.

Christensen (1940) has also investigated the properties of fibrinolysin. He found that it has a plateau-like pH-optimum between pH 5.8 and 9.5. He further proved that it is very thermostabile and even stands being heated to 100° C. without changing.

Tillett and *Garner* (1934) have furthermore shown that the fibrinolysin has a very strong specificity. It does not seem to attack albumin, globulin, casein, gelatin or peptone. The only proteins on

which it has an effect are fibrin and fibrinogen, the latter of which loses its ability to clot with thrombin after incubation with fibrinolysin. Furthermore, the fibrinolysin is type-specific in that fibrinogen from rabbits, for example, is not affected by fibrinolysin from a human-pathogenic haemolytic streptococcal strain with great activity on human fibrin. These authors are also of the opinion that the attack on the protein molecule is slight and that the split products are still of protein nature. The type-specificity has later been analyzed in more detail by *Milstone* (1941), who showed that the explanation lies in the fact that the fibrinolytic activity is linked up with the occurrence of a certain factor in the plasma, present in man but absent in experiment animals. If a solution of this factor is added, fibrin of animal origin is also dissolved.

With a closer study of certain aspects of the properties of the streptococcal fibrinolysin in view, we have tried to work out a method to produce a purified and concentrated preparation from streptococcal broth. The preparation thus obtained has been examined with cataphoresis and in an ultra-centrifuge and was found to be very inhomogenous as to both size and charge of the particles. Attempts to continue the purification have, however, met with great technical difficulties, as the fibrinolysin is strongly bound to a large number of substances in the broth. We have therefore found it best not to aim at a higher degree of purification for the present, but to wait until there are possibilities of cultivating the streptococci on less complicated, possibly synthetic, substrates.

Method for the production, purification and testing of streptococcal fibrinolysin.

Production and purification.

1) A strongly fibrinolytic strain of a haemolytic streptococcus belonging to Lancefield's group A should be chosen. In our experiments a very strongly fibrinolysin-forming strain (called strain G) was used. This strain has, in earlier investigations by one of us (*Winblad* 1941), been compared with a large series of streptococcal strains. Thanks to the least possible sub-cultivation it has kept its high fibrinolysin production, but has, as a laboratory strain, become only faintly toxin-producing.

In order to produce fibrinolysin we sowed the streptococcal strain on 1 litre of vegetable broth containing 1 % peptone, 0.3 % NaCl and 0.05 % dextrose, adjusted to pH 7.6. After 18 hours' growth on this substrate the incubation was broken off by means of cooling with running water. The streptococcal broth was then filtered through a Seitz-filter. We observed when filtering that the fibrinolysin at first tends to be adsorbed at the filter medium, but that, when this has

been saturated, it passes freely through. *Lou* (1941) has proved this by measuring the fibrinolytic powers of the filtrate in early and late fractions, and we were able to confirm his observation. For this reason we chose a small filter surface with a diameter of 3.5 cm and threw away the first 50 ml of the filtrate. Performed in this way, the filtration takes rather a long time, but on the other hand a filtrate containing plenty of fibrinolysin is obtained. Another practicable method is to bring the growth to pH 7.8 by adding bicarbonate in substance. It is then possible to filter through a large filter without loss. Afterwards it is, however, necessary to acidify with acetic acid to pH 5.5 before precipitating the ammonium sulphate.

2) The filtered broth is precipitated by the addition of ammonium sulphate to 70 % saturation. The precipitate is dissolved in about 50 ml aq. dest. and dialyzed over night against running water. No very great purification is obtained by this process, but what it does give is a concentration of the fibrinolysin to a smaller solution volume, which facilitates the subsequent procedure.

3) The solution from 2) is precipitated at 0° C. with 0.85 vol. alcohol. The precipitate is centrifuged in the cold, dissolved in the least possible amount of ice-chilled aq. dest. and dialyzed.

4) The solution from 3) is absorbed with aluminum C_γ at pH 6.5 (about 0.3 gr aluminum gel per litre of the original broth). After washing with aq. dest. it is eluted 3 times, each time with 10 ml/10 phosphate buffer pH 7.8. The eluates are combined and dialyzed.

Table (Preparation XX).

Fraction	Dry weight in mg/ml after dia- lysis	Relative activity	Total activity	Activity per mg dry weight after dia- lysis
Filtered broth culture volume 790 ml	3.7	1	790	0.27
After precipitation with 70% ammonium sul- phate volume 195 ml	6.4	3.2	624	0.50
After precipitation with 85 % alcohol volume 74 ml	5.9	6.4	473	1.1
After adsorption with aluminum C _γ volu- me 36 ml	3.1	12.4	446	4.0
After precipitation with 50 % ammon- ium sulphate volume 6.6 ml	7.3	43	283	5.9
Concentration: 43 times			Yield: 36 %	Purification: 22 times

5) The solution from 4) is precipitated by half-saturation with ammonium sulphate. The precipitate is dissolved in the least possible amount of physiological salt solution and dialyzed against physiological salt solution. The preparation thus obtained is hereafter called purified fibrinolysin.

The table gives a survey of the course of the purification. As is seen from the table, the activity per mg dry weight in the purified fibrinolysin is about 22 times as great as in the original broth.

Measuring of the activity of the fibrinolysin.

The activity of the fibrinolysin has been determined by observing the time required for dissolving a fibrin clot at $+37^{\circ}\text{C}$.

Solutions:

Fibrinogen: Fibrinogen is precipitated from heparin or oxalate plasma from man by the addition of saturated ammonium sulphate solution in an amount corresponding to $\frac{1}{3}$ of the volume of the plasma. After centrifuging the precipitate is dissolved in m/100 phosphate buffer at pH 7.4 to the original volume of the plasma.

Thrombin: Oxalate plasma from man, obtained by adding 1 cc 2 % sodium oxalate solution to 10 cc blood and centrifuging the blood corpuscles, is diluted 10 times with ice-chilled aq. dest. It is then kept iced and bubbled through for 10 minutes with CO_2 . The precipitate thus obtained is centrifuged and dissolved in physiological salt solution to the original volume of the plasma. Some grains of sodium bicarbonate are then added, and also 1 ml 2.5 % calcium chloride solution per 10 ml plasma. A clot is formed within some minutes, which is carefully wound up and pressed out with a glass rod. The remaining solution contains sufficient thrombin for one drop to clot 0.1 cc fibrinogen solution within one minute.

Determination of the activity: In a series of small test tubes are pipetted 0.25 cc fibrinolysin at varying dilutions, 0.2 cc m/15 phosphate buffer at pH 7.5 and 0.1 cc fibrinogen solution. Finally 2 drops of thrombin solution are added to each tube, and the series is put into a water thermostat at $+37^{\circ}$.

All the tubes clot within 1 minute. The tube where dissolution of the clot has occurred within 5—10 minutes is then taken and the time noted. It will now be found that, under the correct experimental conditions, that tube which contains half the amount of fibrinolysin requires almost exactly double the time to liquefy. If, for example, fibrinolysin diluted to $\frac{1}{32}$ dissolves in 10 Minutes its activity will be 3.2.

The mean value should be taken from two determinations lying between 5—20 minutes. For various reasons shorter times gives uncertain results, nor are longer times suitable.

As different thrombin and fibrinogen solutions do not give quite

comparable results, all the fractions from the same preparation have always been determined at the same time. The activity of the broth filtrate has been used as unit, and the activity of the latter fractions has been determined in relation to this. With »total activity« we mean the relative activity multiplied by the volume of the fraction. This has been calculated to enable us to determine the results of the different purification operations possible.

Properties of purified fibrinolysin.

The purified fibrinolysin is obtained as a fairly deep brown solution. Its fibrinolytic activity is so great that, diluted to 1/5,000,000 and under the experimental conditions described above, it can dissolve a fibrin clot in about 10 minutes. The preparation yields positive protein reactions (Biuret, Xanthoprotein and Adamkiewicz-Hopkins). It is precipitated by ammonium sulphate at half-saturation, gives a granular precipitate with trichloroacetic acid, and is not precipitated when boiled in a weakly acid milieu. Molich's reaction on carbohydrate is positive. Its nitrogen content is about 12 %. In a refrigerator, the solution retains a fairly constant activity during 1 month. Diluted to 1:10 the preparation can be injected subcutaneously into the human subject without any perceptible effect. 5 ml of the preparation, injected intravenously into rabbits, do not give any effect.

Summary.

Experiments have been performed aiming at purification and concentration of haemolytic streptococcal fibrinolysin by means of precipitations with absolute alcohol and ammonium sulphate and adsorption on aluminium C_γ in series. The activity per mg dry weight of the fibrinolysin thus treated is increased to 22 times that of the original fibrinolysin. A method of assessing the activity of the fibrinolysin is described. The properties of the purified fibrinolysin are of protein nature. It can, diluted to 1/5,000,000, dissolve a fibrin clot within 10 minutes if no antienzyme is present.

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ÜBER DIE PATHOLOGISCHE ANATOMIE DER SILIKOSE.¹⁾

Von *Hilding Bergstrand*.

(Eingegangen bei der Redaktion am 14. Juni 1944).

Die Silikose wird bekanntlich von einer produktiven Entzündung gekennzeichnet, welche herd- und streifenförmige Gewebsveränderungen sowie schliesslich massige Verdichtungen in der Lunge entstehen lässt. Der herrschenden Ansicht nach sind diese Veränderungen ausschliesslich interstitiellen Charakters. Die eingeatmeten Partikel werden im respiratorischen Parenchym von mononukleären Phagozyten aufgenommen, und das Anfangsstadium ist mithin eine Alveolitis; bald aber wandern die Phagozyten in das interstitielle Gewebe, und ein Teil derselben zerfällt dort auf dem Wege zum Hilus, wodurch Kieseldioxyd frei wird und zur produktiven Entzündung reizt. Die silikotischen Knötchen sollen durch Ansammlung von Kieselsäure in peripheren Lymphfollikeln entstehen, die massiven Herde durch Konfluieren der Knötchen. Gewisse Autoren geben allerdings zu, dass sich — wenigstens bei den rasch verlaufenden Formen von Silikose — auch im Parenchym eine produktive Entzündung abspielt. *Gardner* meint, derartige Staubbpneumonien kämen dann zustande, wenn eine so reichliche Menge von Kieseldioxyd eingeatmet worden ist, dass der normale Reinigungsmechanismus zur Wegschaffung aller Partikel nicht ausreicht.

Eine eingehendere Betrachtung der pathologisch-anatomischen Veränderungen bei *Silicosis pulmonum* scheint jedoch zu dem Ergebnis zu führen, dass auch bei chronisch verlaufenden Formen alveoläre Prozesse sich weit stärker bemerkbar machen, als man es sich im allgemeinen vorstellt.

Bei Elastinfärbung von Schnitten der Bindegewebssepla findet man, dass diese Streifen nicht nur interazinöses bzw. interlobuläres Bindegewebe in vermehrter Menge enthalten, sondern auch eine erhebliche

¹⁾ Vortrag in der Schwedischen Ärztesellschaft am 18. April 1944.

Anzahl von bindegewebig umgewandelten Alveolen. Diese letztere Komponente tritt durch ihren hohen Gehalt an elastischen Fasern manchmal mit erhaltener alveolärer Anordnung hervor, während die erstere elastinfrei ist (Abb. 1).

Aber auch in bezug auf die Entstehung der silikotischen Knötchen kann man die Richtigkeit der meistens verfochtenen Theorien in Zweifel stellen. Es liegt nämlich eine Untersuchung von *Simson* vor, welche bestimmt gegen die Ansicht spricht, dass sich diese Knötchen in den



Abb. 1.

Schnitt aus der Lunge eines Arbeiters, der infolge von Einatmen sublimierter Kieselsäure an Silikose erkrankt war. A interlobäres Septum, an beiden Seiten desselben Auflagerungen von bindegewebig umgewandeltem, kollabiertem Lungenparenchym (B). Das letztere erkennt man an seinem Reichtum an elastischem Gewebe.

Lymphfollikeln bilden. *Simson* hat silikotische Knötchen nach Serienschnitten rekonstruiert und nachgewiesen, dass sie dem Bereich eines Bronchiolus terminalis entsprechen. Bekanntlich besteht die anatomische Einheit der Lunge aus einem Azinus, in den die Atemluft durch einen Bronchiolus terminalis gelangt. Jeder Bronchiolus terminalis teilt sich in zwei Bronchioli respiratorii und jeder einzelne der letzteren in 3—9 Ductuli alveolares, welche sich ihrerseits in 2—3 Alveolarsäckchen verzweigen (Abb. 2). Zu jedem Azinus gehören also ca. 50 Alveolarsäckchen. *Simson* hat nun gezeigt, dass das silikotische Knötchen rings um die Gabelung lokalisiert ist, welche entsteht, wenn sich der Bronchiolus terminalis in Bronchioli respiratorii spaltet. Oft liegen die Knötchen paarweise, zwei Bronchioli terminales entsprechend, welche Äste eines grösseren Bronchus sind. Schon aus dieser Untersuchung geht hervor, dass sich das silikotische Knötchen im Azinusparenchym entwickelt. Es gibt aber auch histologische Anzeichen dafür, dass das Parenchym der Ursprungsort des Knötchens ist.

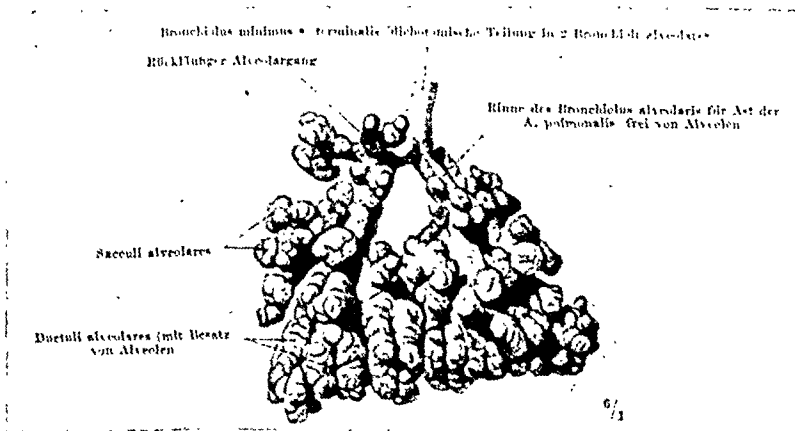


Abb. 2.

Normaler Lungenazinus, zu einem Bronchiolus terminalis gehörendes Parenchym enthaltend. Metallausschuss. Nach *Loeschcke*, Die Morphologie des normalen und emphysematösen Acinus der Lunge. Zieglers Beitr. 68: S. 213, 1921.

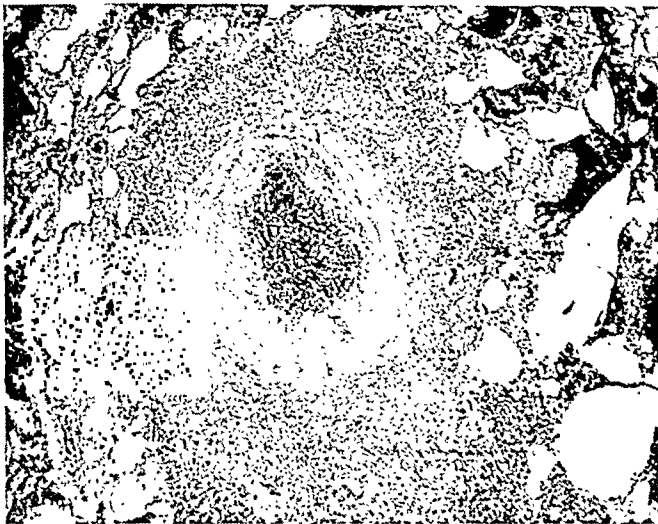


Abb. 3.

Silikotisches Knötchen mit einer stark kohlepigmentierten zentralen Partie, welche eine pigmentfreie, konzentrisch geschichtete Bindegewebskapsel umgibt. Ausserhalb dieser eine breite Zone lockereren, schwarz pigmentierten Gewebes, in welcher sich mittels Elastinfärbung alveoläre Lungenstruktur nachweisen lässt. Das Kieseldioxyd ist an derselben Stelle lokalisiert wie das Kohlepigment, was aus Abb. 4 ersichtlich wird.

Das klassische silikotische Knötchen hat einen recht charakteristischen Bau. Es besteht oft aus einem schwarz pigmentierten Kern von hyalinem Bindegewebe, den eine konzentrische Schicht pigmentlosen, ebenfalls hyalinisierten Bindegewebes umgibt (Abb. 3). Ausserhalb dieser Kapsel befindet sich eine mehr oder weniger breite, stark pig-

mentierte Zone lockereren Gewebes. Durch Studium von Veraschungspräparaten hat sich der Nachweis erbringen lassen, dass die Kieselsäure im Knötchen ganz dieselbe Lokalisation hat wie das Kohlepigment, welches infolgedessen als Indikator für das Kieseldioxyd dienen kann (Abb. 4). Bei Elastinfärbung sieht man weder in dem zentralen Kern noch in der konzentrischen Kapsel elastische Fasern, wohl aber in der peripheren Zone.



Abb. 4.

Verteilung des Kieseldioxyds im silikotischen Knötchen. Im Zentrum reichlich Kieselsäure, desgleichen Kohlepigment. Die zentrale Partie umgibt eine kieseldioxydfreie Bindegewebskapsel mit konzentrisch geschichteten Fasern (aus dem interazinösen Bindegewebe stammend?). Ausserhalb dieser eine kieselsäurereiche, kohlepigmentierte Zone, in der sich elastisches Gewebe mit alveolärer Struktur nachweisen lässt. Veraschungspräparat (Veraschung des Gewebes durch starkes Erhitzen, dann Behandlung mit HCl zur Beseitigung anderer Bestandteile als SiO_2). Nach *Bell, Ferris u. King, J. Path. and Bact.* 51, 1940.

Lokalisation und Umfang des Silikoseknötchens führen leicht zu dem Gedanken, dass die zentrale Partie einem Azinus entspricht, und dass die konzentrische Kapsel durch Wucherung der den Azinus umhüllenden Bindegewebskapsel entsteht. Die äussere schwarz pigmentierte Zone dürfte von Parenchym herrühren, welches zu benachbarten Azini gehört. Durch Verbreiterung dieser Zone können die Knötchen wachsen und schliesslich miteinander verschmelzen. Jeder Bronchiolus respiratorius wird von einer Arterie begleitet, und man muss daher, wenn die vorstehende Deutung richtig ist, im silikotischen Knötchen zwei Arterien sehen können. Dies ist auch oft der Fall. Den von Kapseln umgebenen Knötchen entsprechen wahrscheinlich die scharf begrenzten, sehr dichten Flecke, welche das Röntgenbild bei gewissen Formen von Kieselstaublunge zeigt, beispielsweise bei einer solchen, wie sie bei Eisenerzhäuern in Nordschweden auftritt.



Abb. 5.

Knötchen in der Pleura bei demselben Fall wie in Abb. 3. Das verdickte, elastinlose Pleurabindegewebe wird durch eine elastische Membran von dem übrigen Teil des Knötchens abgegrenzt, welcher aus bindegewebig umgewandeltem elastinhaltigem Lungengewebe besteht.

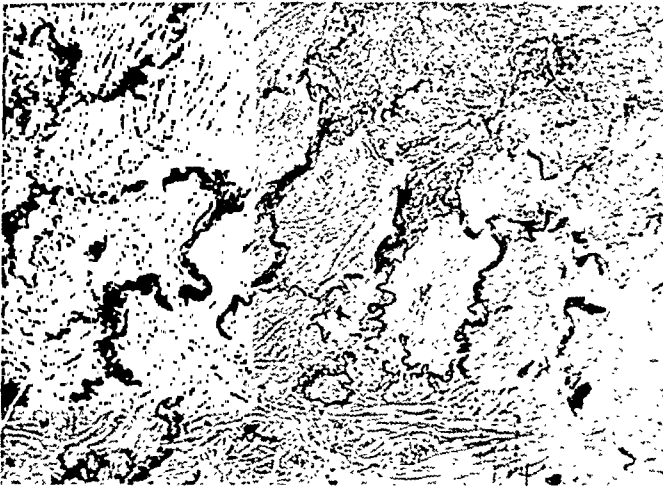


Abb. 6.

Elastingefärbter Schnitt aus einer fibrösen Partie der Lunge eines 54jährigen Mannes, der 37 Jahre in einer Porzellanfabrik gearbeitet hatte und während eines Jahres kurzatmig gewesen war. Trotz vollständiger fibröser Umwandlung des Gewebes macht sich die alveoläre Lungenstruktur durch die elastischen Fasern bemerkbar.

Immerhin haben die Silikoseknötchen bei weitem nicht immer dieses Aussehen. Die konzentrische Schichtung kann fehlen und die äussere Zone aus strahlenförmig angeordneten Fransen bestehen. Derartige Knötchen enthalten häufig nur eine kleinere Menge hyalinen Bindegewebes, und mitunter kann man Reste von Alveolen in denselben entdecken. Das ganze Knötchen hat also ein lockeres Gefüge. Solche Knötchen hat *Gough* bei Kohlenträgern in Cardiff beschrieben; sie entsprechen offenbar den weniger dichten, unscharf begrenzten

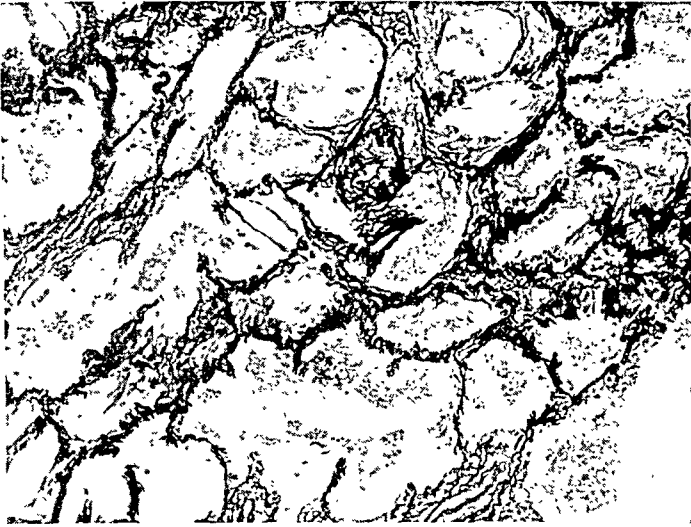


Abb. 7.

Stark verdickte Alveolenwand bei einem Fall von Silikose im dritten Stadium. Lumina der Alveolen mit mononukleären Zellen gefüllt, welche Kohlepigment und Fetttropfchen enthalten. Silberimprägnierung.

Flecken, die man im Röntgenbilde bei gewissen Silikoseberufen sieht.

Die Fleckigkeit des Röntgenbildes hängt jedoch sicherlich nicht nur mit dem Vorkommen silikotischer Knötchen zusammen, sondern auch mit der ungleichmässigen Verteilung der Bindegewebsvermehrung in interazinösen und interlobulären Septa, in welchen Lymphgefässe verlaufen, die das Kohlendioxyd dem Hilus zuführen.

Auch das Aussehen der silikotischen Pleuraknötchen spricht in gewissem Masse dafür, dass dieselben einem Azinus entsprechen. Sie sind nämlich kegelförmig, im Schnitt dreieckig mit der Basis in der Pleura. Die Basis selbst wird von Pleurabindegewebe gebildet, welches durch eine elastische Linie von dem übrigen, aus elastinhaltigem, bindegewebig umgewandeltem Lungengewebe bestehenden Teil des Knötchens abgegrenzt wird (Abb. 5).

Bei gewissen Formen von Kieselstaublunge offenbaren auch die voluminösen, massiven Bindegewebsmassen, welche das dritte Stadium kennzeichnen, ihre Genese durch einen produktiven pneumonischen Prozess, indem Elastinfärbung ein alveoläres Elastinnetz inmitten des

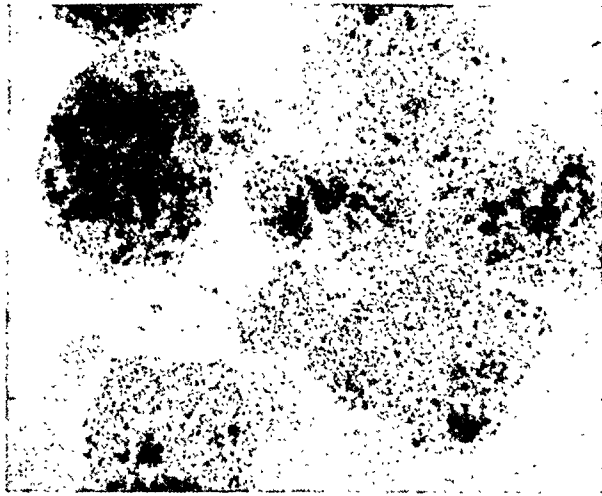


Abb. 8.

Mit Sudan gefärbter Schnitt aus einer Kieselstaublunge (drittes Stadium). Mononukleäre Phagozyten, scharf hervortretende, teilweise kantige Kohlepartikel und schwach abstechende runde Fettröpfchen enthaltend.



Abb. 9.

Rundliche, fibröse, konzentrisch geschichtete Bindegewebsherde rings um Tropfen von 12 Jahre vorher injiziertem Kampferöl. Im Zentrum sieht man bei zwei Herden Lücken nach Auslaugung der Fettsubstanz.

hyalinisierten Bindegewebes zutage bringen kann (Abb. 6). Das Aussehen des Parenchyms in der Umgebung der Bindegewebsmassen gibt Aufschluss über die Entstehung derselben. Man findet nämlich durch Bindegewebsneubildung stark verdickte Alveolenwandungen (Abb. 7) sowie ganz oder teilweise kollabierte Alveolen.

In noch einer wichtigen Beziehung ist die Pathogenese der Lungen-

silikose umstritten, nämlich hinsichtlich der Wirkungsweise der Kieselsäure.

Im allgemeinen ist man der Ansicht, die Lungenfibrose sei eine direkte Folge der Reizung durch Kieseldioxyd, und experimentelle Untersuchungen scheinen zu ergeben, dass die Reizung um so stärker ist, je kleiner die Partikel sind. Es gibt indessen eine andere Theorie, laut welcher die produktive Entzündung eine Reaktion auf die beim Zerfall der Monozyten frei werdenden Lipoiden sein soll. *Fallon* hat nachgewiesen, dass die Lungen bei Kaninchen, bei denen eine Silikose experimentell erzeugt worden war, viel lipoidreicher sind als normalerweise, und dass sich mit den extrahierten Lipoiden ganz dieselbe Reaktion hervorrufen lässt wie mit Kieselsäure. Schon die histologische Untersuchung macht übrigens ersichtlich, dass die silikotischen Lungen oft ausserordentlich lipoidreich sind. Nach Sudanfärbung sehen grosse Partien der Lungen infolge ihres Reichtums an lipoidhaltigen Monozyten vollständig rot aus (Abb. 8), und es ist seit langem bekannt, wie sehr die Lipoiden zu produktiver Entzündung zu reizen vermögen. Rings um einen mehrere Jahre in der Haut liegenden Öltropfen kann sich ein Knötchen entwickeln, welches aus hyalinisierten, konzentrisch geschichteten kollagenen Fasern besteht; die Ähnlichkeit eines derartigen Gebildes mit einem Silikoseknötchen ist eine überaus grosse (Abb. 9). Dieser Gedanke ist also nicht ohne weiteres von der Hand zu weisen.

Schliesslich sei erwähnt, dass Kieseldioxyd eine Substanz ist, welche jeder Mensch einatmet, und dass demnach das von uns als *Silicosis pulmonum* bezeichnete Krankheitsbild nur ein relativer Begriff ist, dessen Abgrenzung konventionell werden muss. Dieser Umstand ist geeignet, bei der versicherungsmedizinischen Gutachtertätigkeit Schwierigkeiten zu bereiten.

THE PATHOGENESIS OF ACUTE POLIOMYELITIS. HISTOLOGIC EXAMINATION OF THE PERIPHERAL SYMPATHETIC NERVOUS SYSTEM AND THE OLFACTORY BULBS.

Erik Waaler M.D.

(Received for publication June 30th, 1944).

The poliomyelitis virus is a neurotropic virus and it is the general belief by all modern investigators that the virus in the nervous system travels by the way of the axis cylinders. The older theories, maintained by *Harbitz* and *Scheel* and others, that the virus spreads along the lymphatics and the spinal fluid are discarded.

Whereas there is, at present, agreement as regards the spread of the virus in the nervous system, the mode of invasion into the nervous system is still disputed. Most authors believe that the port of entry of the poliomyelitis virus in man is the nasal membrane via the olfactory nerves to the olfactory bulbs. Another theory, which has few advocates, regards the intestinal tract as the port of entry.

The theory of invasion through the digestive canal has been accepted by *Wickmann* and in later years by *Kling* and co-workers. In America the intestinal route of invasion has been strongly supported by *Toomey*. He finds in numerous publications both clinical and experimental evidence supporting this theory. According to *Toomey* the virus from the intestinal mucosa follows the unmyelinated fibres of the sympathetic system to the spinal cord. The bulbar cases develop as a result of invasion through the 10th nerve, in few instances through the 7th nerve. In the feces of poliomyelitis patients *Toomey* has found a toxic product, »enteric toxin«, which makes the experimental animals more sensitive to the virus. In the »natural« infection in man this enteric toxin is presumed by *Toomey* to make contact possible between the virus and the sympathetic fibres in the intestinal wall.

Monkeys that have been infected through the nasal mucosa show specific histologic changes in the olfactory bulbs. (*Sabin* and *Olitsky*,

Howe and Ecke). Similar changes have not been demonstrated in human poliomyelitis cases. *Harbitz* and *Scheel* have examined the olfactory bulbs in some cases and found them normal. *Sabin* in 1940 reported 83 cases studied in the literature, and added 10 cases of his own in which the bulbs were without definite changes. These findings reason against but do not definitely rule out the olfactory bulbs as the port of entry in man.

If Toomey's theories were correct, one might expect to find histologic changes in the peripheral sympathetic system in human poliomyelitis cases. Usually one does not pay very much attention to the sympathetic system in poliomyelitis. *Pette*, *Demme* and *Környey* did not find any changes in the sympathetic ganglia in a monkey infected by the intestinal route, but another monkey which was infected by intravenous injection of virus showed alterations in the autonomic ganglia. *Pette* states in a monograph published in 1942 that the peripheral sympathetic system as a rule is without anatomical changes in poliomyelitis.

Mouriquand, *Dechaume*, *Sédallian* and *Morin* in 1930 reported a case in a girl with marked intestinal symptoms in the prodromal stage of the disease. At autopsy they found ulceration and inflammatory changes in the intestinal mucosa, degeneration of the ganglion cells in the intestinal wall and proliferation of the satellite cells. Affection of the nerve cells and neuronophagia were also demonstrated in the coeliac ganglion. In experimental poliomyelitis, using intraperitoneal inoculation, the same authors found similar changes in the peripheral sympathetic system. They claim that these findings support the theory of intestinal invasion, and indicate that the virus travels along the sympathetic fibres to the central nervous system.

In a series of cases of acute poliomyelitis in 1941—1942 and 1943 we have studied the peripheral sympathetic system, and the results of these investigations are reported in this paper. We have paid particular attention to the autonomic ganglia of the sympathetic trunk and to the coeliac ganglion and the vagus nerve with ganglia. In view of the findings of the French authors we have also studied the sympathetic cells of the intestines. The results of the examination of the olfactory bulbs are also reported.

Material and Methods.

Altogether 42 cases of poliomyelitis were examined, 21 males and 21 females. In 15 cases the sympathetic system was studied, all the cervical ganglia, 4—6 thoracic, 2—4 lumbar ganglia on both sides and the coeliac ganglia were removed. In seven cases the vagus nerve with the ganglion nodosum on both sides was examined. The sections were stained with hematoxylin and eosin, with thionin and according to *Bodian's* silver method.

From the intestines, specimens were removed from the upper and lower part of the ileum in 16 cases. The sections were stained with hematoxylin and eosin, with thionin and in a few cases with Bodian's silver method.

In 19 cases the olfactory bulbs were removed in toto, as a rule after the brain had been fixed in formalin for some days. The bulbs were divided in two by frontal section. Both parts were embedded and 10 to 20 sections were stained with thionin in each case.

In the central nervous system several sections from the cord, spinal ganglia, medulla and pons, thalamus and gyrus centralis anterior were examined, so that the distribution of the lesions in each case was revealed.

In order to get personal experience as regards the normal variations, the olfactory bulbs, the intestines and the peripheral sympathetic system were studied in a series of control cases. These examinations proved to be very important particularly with regard to the sympathetic ganglia where apparently the extent of normal variations is far greater than is usually supposed. As control cases we selected individuals who had died from accidents, suicide and various acute diseases, altogether 17 cases.

Olfactory Bulbs.

Of the 19 cases two were of the bulbar type and 17 were spinal cases. The structure of the bulbs was regularly quite loose, probably due to edema. There were no degenerative changes in the nerve cells. No diffuse or focal cellular infiltration and no perivascular infiltration were present. In some cases there was a slight infiltration of lymphocytes in the meninges, but not more than could be found elsewhere in the meninges covering the brain. The olfactory bulbs in these 19 cases were thus completely normal.

The Intestines.

Altogether 16 cases were examined, three of the bulbar, and 13 of the spinal type. In some cases there had been moderately severe abdominal symptoms with pain, loose stools or constipation; but in no case had the intestinal symptoms been very marked.

The ganglion cells in the plexus myentericus showed up well in the thionin sections, varying much in size and shape. Some were round, others angular, with irregular indentations. As a rule, the cells were pale but quite distinct, with dense, finely granular Nissl substance, and the position of the nuclei was often eccentric. (Fig. 1). Degenerative changes which could be described as »severe cell change« and neuronophagia were nowhere to be found. In many cases, however, the cells were irregular with vacuolated cytoplasm (Fig. 2), and the satellite cells stood out very prominently, but did not show proliferative changes. Also among the controls were found vacuolated

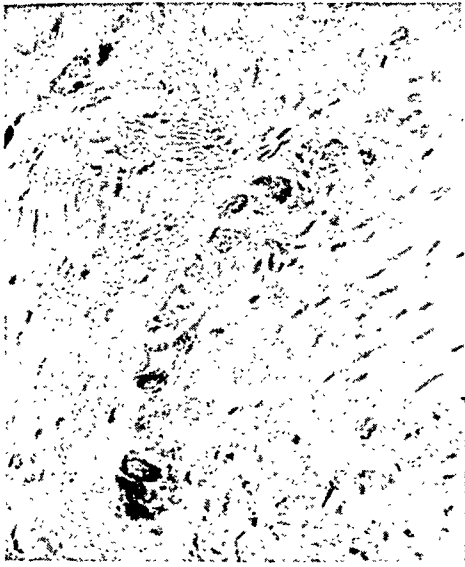


Fig. 1.

Ganglion cells in the intestine. The cells fairly well preserved. Thionin.



Fig. 2.

Ganglion cells from the intestine. The cells vacuolated with pycnotic nuclei. Thionin.

and irregular cells as those seen in Fig. 2 and the cells with this appearance are therefore not interpreted as degenerated cells. On scrutiny of the control material, the morphology of the ganglion cells showed no features distinguishing them from those of the poliomyelitis cases. The findings in the poliomyelitis cases are therefore regarded as being within the range of normal variation.

In no case was ulceration and necrosis of the mucous membrane observed, and there were no changes which could be considered as unquestionably inflammatory. The fairly plentiful lymphoid tissue often noted was not remarkable considering the high proportion of children and juveniles in this material.

The Peripheral Sympathetic Ganglia and the Vagus Nerve.

Of the 15 cases examined, 14 were of the spinal, and one of the bulbar type. These cases are tabulated in their entirety (table 1) the controls likewise (table 2). At the first scrutiny of the sympathetic ganglia, it is difficult to be sure of the morphologic qualities of the cells, and it is remarkable how much they vary in staining and shape from one visual field to another. Hence, as already indicated, the great need for control material in order to distinguish between the normal and the pathologic. The nucleus is very often situated in the periphery, and the Nissl substance is unevenly distributed, the largest and most dark granules being concentrated in the periphery, whereas the central parts of the cells are fairly light. This state of affairs

is of no pathologic significance as far as the sympathetic cells are concerned, — a point emphasized in the literature (*De Castro, Massig* and others). Now and then one sees fairly homogeneous, small, dark cells with ill-defined nuclei, and pale, faintly stained cells, and yet other cells whose cytoplasm is rather finely vesicular. Around the pale cells and the finely vesicular cells there are often to be seen remarkably well-defined satellite cells to which no special importance need be attached. Other cells again show irregular surface indentations.

Table 1.
Changes in the Sympathetic Ganglia in Poliomyelitis.

Number	Age	Sex	Type of clinical picture	Duration	Severe cell change	Neuro-nophagia	Focal or diffuse cellular infiltration	Perivascular lymphocyte infiltration
1.	8 years	F.	Spinal	8 days	+?	+?	+	+
2.	4 "	M.	Spinal	6 "	0	0	0	+(few)
3.	14 "	F.	Spinal	4 "	0	0	+(few)	+(few)
4.	18 "	M.	Spinal	6 "	+	+	+	+
5.	16 "	M.	Spinal	3—4 weeks	0	0	+(few)	+
6.	38 "	M.	Spinal	3 days	+?	+?	+	+
7.	21 "	M.	Spinal	2—3 "	+?	+?	+	+
8.	38 "	M.	Spinal	1½ "	+	+	0	0
9.	34 "	F.	Spinal	10 "	0	0	0	+(few)
10.	8 "	F.	Spinal	5 "	0	0	0	0
11.	38 "	M.	Spinal	14 "	+	+(few)	+(small)	+(one)
12.	20 "	F.	Spinal	7—8 weeks	0	0	0	0
13.	13 "	F.	Spinal	8—10 days	0	0	+(few)	+
14.	19 "	F.	Bulbar	4 "	0	0	0	0
15.	15 "	M.	Spinal	4 "	0	0	+(one)	0

Some of these changes are possibly post-mortem, as held by *Masig*, but other changes may possibly reflect injuries to the cells in the course of the acute disease responsible for the patient's death or, possibly due to agonal influence. The control material suggests that these slight changes in the ganglion cells are so common that they hardly deserve notice. Our first impression was that these changes were most common in patient's dying of an acute infection, but the personal equation makes such an impression unreliable.

Perivascular lymphocyte infiltrations are also often to be found in normal ganglia, appearing as a cuffshaped cape embracing certain small vessels. This phenomenon may be noted in young persons whose

death is due to some accident (see cases 2, 3 and 17 in table 2), and is regarded as a normal occurrence. Furthermore, no importance has been attached to small and isolated nodules of lymphocytes.

On the other hand, larger, ill-defined cell infiltrations have been noted as pathologic and deserving of discussion when there are several of them (Fig. 3), and this is also the case with certain cell changes which can be classified as »severe changes« (»Schwere Zellerkrankung« of Nissl) (Fig. 4 and 5). These changes consist of vesicle formation within the cells, the breaking down of Nissl substance and

Table 2.
The Sympathetic Ganglia in the Control Material.

Number	Age	Sex	Diagnosis	Severe cell change	Neurophagia	Focal cellular infiltration	Perivascular infiltration of lymphocytes
1.		M.	Carcinoma of the prostate.....	0	0	0	0
2.	30 years	M.	Suicide	0	0	0	+
3.	50 »	M.	Suicide	0	0	+(few)	+(few)
4.	12 »	M.	Syphilis	0	0	0	0
5.	1 »	M.	Acute gastroenteritis.....	0	0	0	0
6.	53 »	F.	Peritonitis.....	0	0	0	0
7.	70 »	F.	Fracture of the collum femoris	0	0	0	+(several)
8.	43 »	M.	Heart block	0	0	+(small)	+
9.	11 $\frac{1}{4}$ »	M.	Combustion	0	0	+(one)	0
10.	15 »	F.	Diphtheria	+	+	+(few)	+(few)
11.	41 »	M.	Syphilis	0	0	0	0
12.	16 »	F.	Diphtheria	0	0	0	0
13.	15 »	F.	Acute hepatitis	0	0	0	0
14.	27 »	F.	Encephalitis	0	0	0	0
15.	9 »	M.	Diphtheria	0	0	0	0
16.	6 »	M.	Combustion	0	0	0	0
17.	6 »	F.	Accident Fracture of the skull..	0	0	0	+(few)

lysis of the cells. Thickening of the neuro-fibrillae and vacuole development in the cells in Bodian section are also looked upon as a severe cell change. These considerable cell changes are accompanied by definite proliferative changes in the satellite cells with neuronophagia. These changes were found in only one of the controls, a case of diphtheria, and are interpreted as signs of definite pathologic processes.

Lastly, in the silver preparations, there is a phenomenon difficult of interpretation, — tuberos swelling on the nerve fibres (Figs. 7 and 8). These swellings can be seen close to the ganglion cells on the dendrons, and they presumably reflect some injury to the ganglion

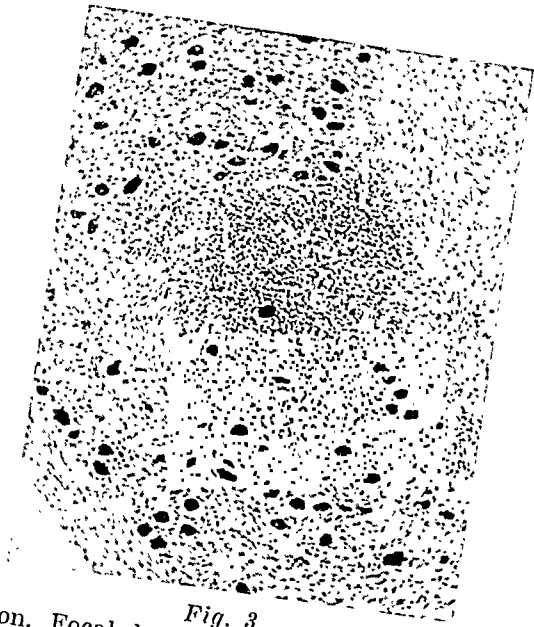


Fig. 3.
Sympathetic ganglion. Focal lymphocytic infiltration. The ganglion cells within this area small and shrunken, but no definite severe cell change can be seen. Case No. 6. Thionin.

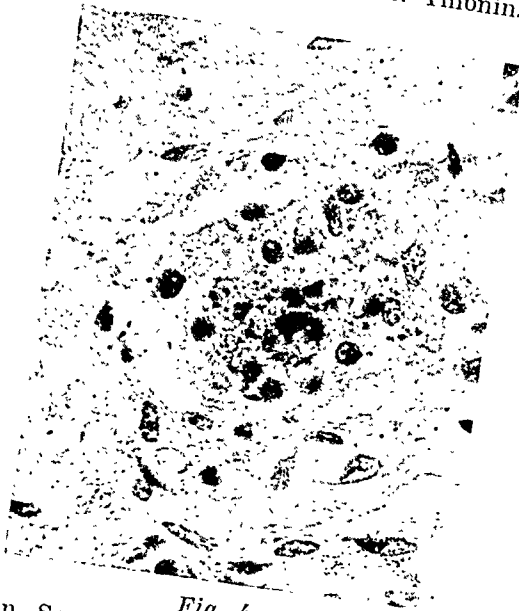


Fig. 4.
Sympathetic ganglion. Severe cell change and neuronophagia. Case No. 8. Thionin.

cells. In the present study these swellings appeared for the most part on the nerve fibres passing through the ganglia, their perikaryon apparently not being in the ganglion itself. It would be natural to

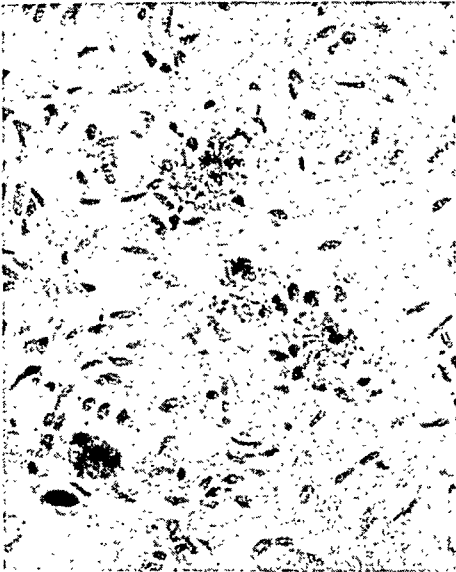


Fig. 5.

Sympathetic ganglion. Severe cell change and neuronophagia. Case No. 8. Thionin.

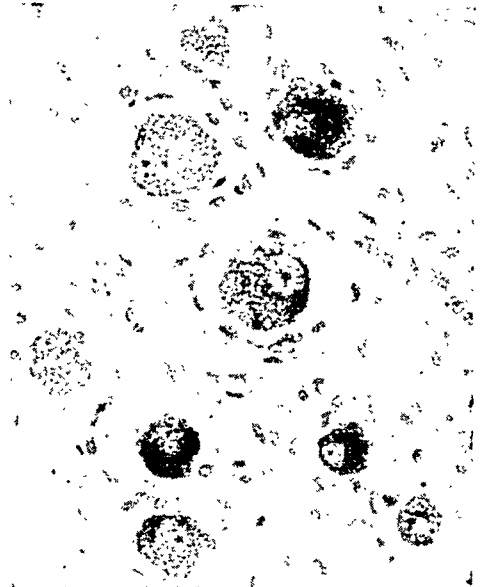


Fig. 6.

Sympathetic ganglion. Normal control. Thionin.

suppose that these swellings, which to a certain extent make the nerve fibres look like a string of pearls, are the outcome of injury to the ganglion cells. Yet the same condition was, to a certain extent, also observed among the controls. To be sure, these swellings were more frequent and prominent among the poliomyelitis cases, but the difference was not very great. These findings have been put on record, but at present their significance is not clear.

In table 1 are three cases without degenerative changes or cell infiltrations (cases No. 10, 12 and 14). In six cases there were a few slight perivascular infiltrations and a few small lymphocyte infiltrations (cf. No. 2, 3, 5, 9, 13 and 15). All these cases are regarded as negative. It is not certain that these findings are pathologic, and it is at any rate doubtful if the slight changes observed in the six cases are due to the poliomyelitis. The five cases in which there were more prominent changes will be discussed more fully.

Case No. 1, autopsy 131/41. An 8-year old girl fell ill on May 28 with shivering and headache. The paresis of both arms noted on May 31 spread to the chest and neck. The respiratory disturbances increased, and death occurred on June 5. The post-mortem examination was made ten hours later.

The nervous system showed considerable damage to the spinal cord, with almost complete disappearance of the anterior horn cells in the cervical, thoracic, and lumbar sections. There was profuse

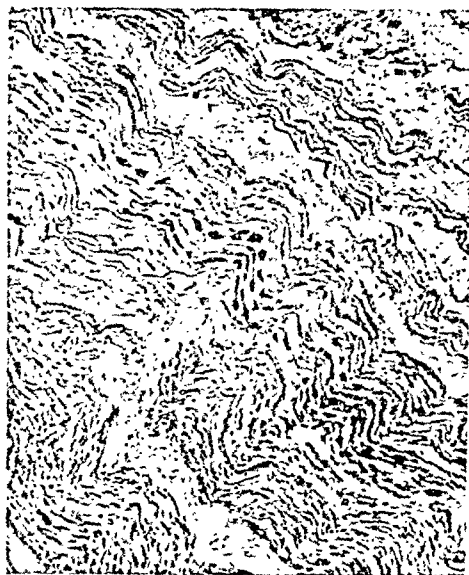


Fig. 7.

Sympathetic ganglion. Tuberos swellings of the nerve fibres. Bodian silver impregnation.

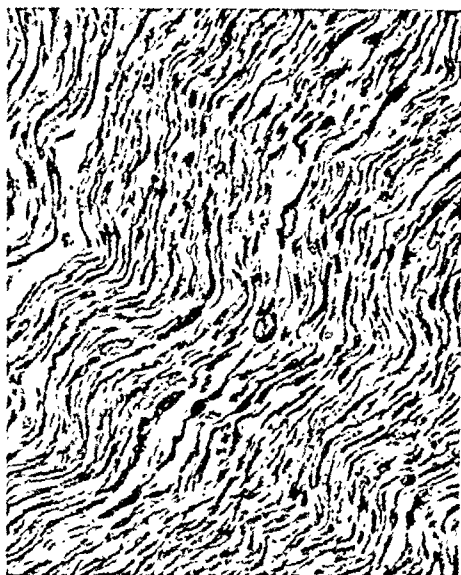


Fig. 8.

Sympathetic ganglion. Tuberos swellings of the nerve fibres. Bodian.

infiltration with granular cells, with neuronophagia and perivascular lymphocyte infiltration. There was also considerable damage done to the medulla oblongata, with neuronophagia in the nucleus vestibularis med. and in the nucleus ambiguus. Likewise in the pons there was neuronophagia, and scattered glial infiltrations were observed

in the thalamus. There was also glial infiltrations of the gyrus centralis anterior, and perivascular infiltration of the grey and white matter. The spinal ganglia were not examined.

The sympathetic nervous system. (The cervical, thoracic, and lumbar ganglia and the coeliac ganglion). There were several densely packed perivascular lymphocyte infiltrations, and a certain number of focal cell infiltrations unrelated to the vessels and ganglion cells. Several of these focal lymphocyte infiltrations were small, but there were also a few which were somewhat larger, less well defined infiltrations resembling the one reproduced in fig. 3. Within such an area, a certain number of ganglion cells seemed to have disappeared. Further, within a cervical ganglion there was a nodule of cells centring about a damaged ganglion cell which had almost undergone neuronophagia. As this picture was not, however, quite convincing, the case is referred to in the table with a question mark.

The overwhelming majority of the ganglion cells showed a finely distributed Nissl substance. Yet there were also to be seen certain pale cells, and others with angular, irregular outlines, unevenly distributed Nissl substance, and defective staining of the nuclei.

The Bodian preparations showed a uniformly beautiful impregnation of the ganglion cells with their offshoots. The silver preparations showed no definite cell changes. Here and there tuberos swellings were seen on nerve fibres situated in the periphery of the ganglion. This observation was, however, very rare, and there was no demonstrable fragmentation. No tuberos swelling of the dendrites close to the cells was seen.

Case No. 4, autopsy 199/41. A man, aged 18, suffered from headache on August 14. He had to take to his bed next day with pain in his legs. On admission to hospital on August 17, both his legs were paralysed. The paralysis increased, and death occurred on August 20. Autopsy 12 hours later.

The microscopic examination showed almost complete destruction of the anterior horns in the lumbar, thoracic, and cervical parts of the medulla and to a certain extent, injury to the cells in Clarke's column. The medulla oblongata and pons showed only scattered neuronophagias and glial infiltrations. The gyrus centralis anterior was also involved. The spinal ganglia showed several damaged cells forming nodules, and focal infiltration of the satellite cells.

The sympathetic nervous system. The cervical and thoracic ganglia and the coeliac ganglion were examined, and hyperaemia, several scattered perivascular infiltrations and small, well-defined lymphocyte infiltrations were found. On the whole, the ganglion cells were in good condition, the finely granular Nissl substance being easily visible, and the nuclei with nucleoli well-defined. The outlines of certain cells were, however, completely blurred, with clumping of the Nissl substance and disappearance of the nuclei. Certain cells had completely disappeared as a result of neuronophagia, only traces

of ganglion cells being left in the nodules of cells. Yet it was rare to find a few scattered cells which had undergone »severe change« and which showed incipient or completed neuronophagia in the thoracic ganglia, the coeliac ganglion and, at one point, in the cervical ganglia. The Bodian preparations showed satisfactory staining of the cells and their offshoots. A few, small, spindle-shaped swelling of nerve fibres as they passed through the ganglia were demonstrable. Bud-dings on the dendrites were not seen. The changes observed in this case may thus be said to be definite and pathologic, but very slight. The cell infiltration and damage to the ganglion cells were less marked than was the case with the spinal ganglia in which the changes may be said to be moderate.

Case No. 6, autopsy 204/41. A man, aged 38, fell ill on August 24 with headache and rigidity of the neck. His arms became paralysed on August 26. He became rapidly worse, and death occurred on August 27. Autopsy on the same day, seven hours after death.

There was almost complete destruction of the anterior horns in the lumbar, thoracic, and cervical parts of the medulla. There was also partial necrosis with neuronophagia of the cells in Clarke's column. There were slight changes in the medulla oblongata and pons, and some injury to the nucleus of the hypoglossal nerve and the facial nucleus. There were also scattered glial infiltrations. There were no changes in the gyrus centralis anterior. In the spinal ganglia were several damaged cells, with neuronophagia, proliferation of the satellite cells, and infiltration with leucocytes.

The sympathetic nervous system. The cervical, thoracic, and lumbar ganglia, the coeliac ganglion and the vagus nerve were examined. The cervical, thoracic, and lumbar ganglia showed no cell infiltrations of any kind, and nowhere could definite »severe cell change« in the ganglion cells be noted, although there were many pale cells and others whose outlines were defective and whose nuclei were ill-defined. The coeliac ganglion showed in several sections a quite large, ill-defined infiltration with cells consisting to some extent of lymphocytes (Fig. 3). Within this area are several small shrunken cells without nuclei, and it is possible that a few small cells are missing, having been replaced by infiltrating cells. It is not clear whether we are confronted here by a primary cell destruction with consequent cell infiltration or by a cell infiltration which is the primary phenomenon, and none of the many sections scrutinized could settle this point. In one visual field, however, it seemed likely that the cell in question had undergone neuronophagia. However, the majority of the ganglion cells were well preserved, showing no hint of degenerative changes.

The vagus showed slight irregularity in the impregnation of nerve fibres in Bodian preparations, — probably the result of injury to the nerve on its removal from the body. The Bodian preparations showed

no buddings on the dendrites, and the longer offshoots also showed no changes. On the other hand, the fibres of the vagus were little swollen and frayed out as a result, no doubt, of artificial influences.

Thus the changes found in this case concerned only the coeliac ganglion and were very slight: no definite neuronophagia was demonstrable. Probably the infiltration observed was connected with the poliomyelitis, but the findings are open to doubt.

Case No. 7, autopsy 206/41. A man, aged 21, suffered from paralyses for two to three days. His death occurred on August 28, and the post-mortem examination was made 48 hours later.

There was practically complete destruction of the anterior horns of the lumbar, thoracic, and cervical sections of the medulla examined. The medulla oblongata and pons showed only slight changes, with some injury to the nucleus ambiguus and the dorsal vagus nucleus. There were also scattered glial infiltrations, with an extensive mesenchymal process. The brain-stem showed mesenchymal changes. The gyrus centralis anterior was not examined. The spinal ganglia showed a quite extensive process, with several nodules of satellite cells and ganglion cells more or less damaged.

The sympathetic nervous system. The cervical, thoracic, and lumbar ganglia, the coeliac ganglion and the vagus with the ganglion nodosum were examined. The structure of the ganglion cells was, to a certain extent, considerably blurred, probably because of the length of the interval between death and the post-mortem examination. Nowhere could cell infiltrations be found in the coeliac ganglion or the lumbar ganglia, whereas several small and medium-sized infiltrations were seen in the cervical and thoracic ganglia. Some of these infiltrations were perivascular, whereas others showed no relationship to vessels or ganglion cells. In the neighbourhood of a few infiltrations, ganglion cells were seen presenting changes which could probably be described as »severe cell change«. At two points, hypertrophy of satellite cells, with what was probably neuronophagia, could also be seen. The ganglion nodosum showed changes similar to, but much less marked than, those found in the spinal ganglia. There were only a few damaged cells with hypertrophy of satellite cells, but no complete neuronophagia was observed. The process would thus seem to be more recent than that noted in the spinal ganglia. The Bodian preparations showed no definite changes in the nerve fibres.

The changes in the sympathetic ganglia must therefore be regarded as quite slight, and it is not absolutely certain, though it is perhaps likely, that they were due to the poliomyelitis.

Case No. 8, autopsy 222/41. A man, aged 38, fell ill in the small hours of September 18 with pain in the back and legs and pareses of the legs and right hand. He was admitted to hospital on September 19, and he died on the same day of paralysis of the respiratory system.

The post-mortem examination was made 15 hours later, also on September 19.

The microscopic examination of the central nervous system showed a very extensive process, with complete destruction of the anterior horns of the spinal medulla in the lumbar, thoracic and cervical sections examined. The cells of Clarke's column were also destroyed. The process was, to a certain extent, in the stage of polymorphnuclear

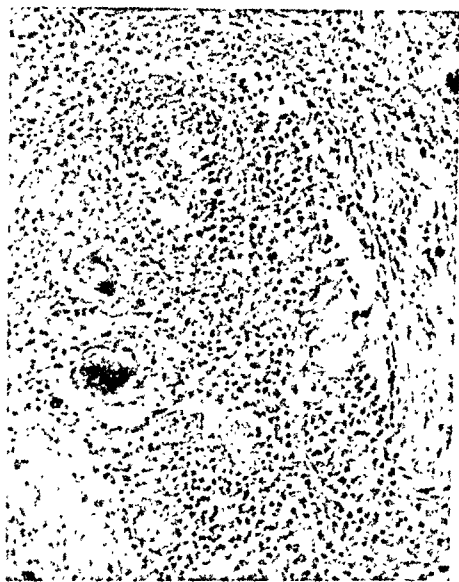


Fig. 9.

Spinal ganglion. Severe cell change and neuronophagia. Case No. 8. Thionin.

leucocytes. There was also fairly extensive damage to the medulla oblongata and pons, the nucleus of the trochlear nerve, the motor nucleus of the trigeminal nerve and, in particular, the nucleus tractus solitarii. The process was here in large measure at the stage of polymorphnuclear leucocytes. The thalamus showed extensive mesenchymal changes, and in the gyrus centralis anterior were several glial infiltrations, but there was no neuronophagia. The spinal ganglia showed an extensive process, with numerous damaged cells replaced by proliferating satellite cells.

The sympathetic ganglia. The cervical and thoracic ganglia, the coeliac ganglion and the vagus were examined. In the coeliac ganglion in particular there were several cells showing »severe change«. The cells were large and swollen, with blurred outlines and large clumps in what was left of the cytoplasm; some of the cells had broken down almost completely. About these cells there was considerable proliferation of the satellite cells, with incipient neuronophagia of the damaged ganglion cells, (Figs. 4 and 5). These changes were demonstrable in several visual fields, yet most of the ganglion cells showed no definite

degenerative changes. At no point could perivascular lymphocyte or other cell infiltrations be seen. The Bodian preparations showed no definite changes. The fibres of the vagus showed slightly irregular thickening, with spindle-shaped distension, but it is probably that these changes were within the range of normal variation. Thus, though the changes were quite definite, they were nevertheless not considerable. The destruction in the spinal ganglia was more extensive. To

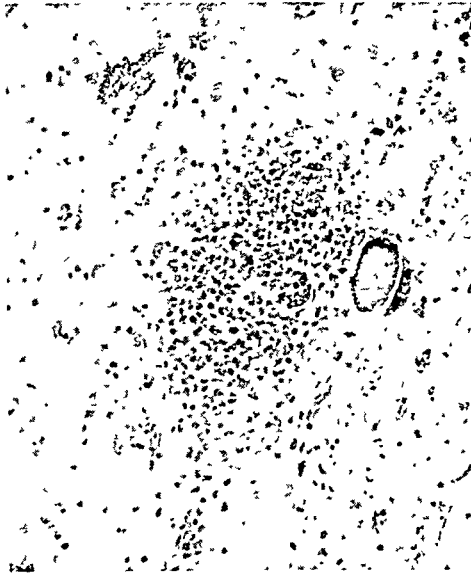


Fig. 10.

Clark's column. Severe cell change and neuronophagia. Case No. 8. Thionin.

judge by the stage reached, the process in the spinal ganglia would seem to be more advanced and probably older than that in the sympathetic ganglia. (For the purpose of comparison there is reproduced in fig. 9 a picture of the findings in a spinal ganglion of the same patient, and in fig. 10 the changes in Clarke's column). There can be no doubt that the changes found in the sympathetic ganglia were due to the acute disease from which the patient had recently suffered.

Case No. 11, autopsy 250/41. A man, aged 38, suffered from headache and pain in the lower limbs on October 12. On October 16, his legs became paralysed, and next day paralysis of the respiration set in. He died on October 26, the postmortem examination being conducted on the following day, 26 hours after death.

The histologic examination of the central nervous system showed practically complete destruction of the anterior horns of those sections of the cervical, thoracic and lumbar medulla which were examined. The infiltrating cells were scavenger cells. The cells of Clarke's column were also damaged on one side, certain cells showing neuronophagia.

There was slight involvement of the nucleus ambiguus and nucleus tractus solitarii, and the substantia nigra also showed neuronophagia. There were mesenchymal changes in the thalamus, and the gyrus centralis anterior showed some neuronophagia with glial infiltration. The spinal ganglia showed only slight changes, — a few small focal lymphocyte infiltrations and slight degenerative changes in the ganglion cells. A few cells were replaced by nodules of satellite cells.

The sympathetic nervous system. Ganglia from the cervical, thoracic and lumbar plexus, as well as the coeliac ganglion, were examined. There were a few lymphocyte infiltrations, partly perivascular, partly focal, without any definite relationship to the vessels or the ganglion cells. Most of the ganglion cells showed no degenerative changes, and only a few cells were slightly frayed out with fairly large satellite cells which did not, however, show definite proliferation. A nodule of cells, in the centre of which were remains of a ganglion cell, was discovered. This represented practically complete neuronophagia. Here, too, the process in the sympathetic ganglia seemed to be of more recent date than that in the other parts of the nervous system.

The Bodian sections showed no definite changes.

The changes in the sympathetic ganglia were also in this case quite slight, but can well be associated with the acute disease from which the patient had recently suffered.

There was also in this material a case of the bulbar type (No. 14). Here there was a very extensive process in the medulla oblongata and pons, whereas the process was of more recent date and more scattered in the spinal medulla. The sympathetic ganglia showed no changes in this case in which the vagus with the ganglion jugulare, the ganglion nodosum and the ganglion petrosum was examined. Here and there in these ganglia were cells with »severe change« and hypertrophy of the satellite cells. Most of the cells were intact, and there was no demonstrable neuronophagia. Here, too, the process seemed to be of more recent date than that in the central nervous system.

Discussion.

In this collection of 19 cases, the examination of the bulbus olfactorius again yielded wholly negative results with regard to histologic changes. Neither was there found in this material any support for the assumption that the infection in man follows the same route as that in intranasally infected monkeys. To be sure, it is conceivable that the virus takes this course in man all the same, for some reason or other giving rise to no changes in the process. But this explanation does not seem plausible. In animal experiments it is assumed that

histologic changes occur in those parts of the nervous system which have been reached by the virus, and it is improbable that matters are different in this respect in man. It is conceivable that the changes in man are so slight that they cannot be revealed by our histologic methods, or that the changes in the bulbus olfactorius are so completely superficial and rapidly transient that normal conditions are quickly restored. But this also does not seem very likely considering the similarity of the anatomic changes in the poliomyelitis of monkeys and man.

The examination of the nerve plexus of the intestinal tract has also proved quite negative. In the case reported by Mouriquand, Dechaume, Sedaillon and Morin, the remarkable prominence of the symptoms referable to the intestinal tract was so striking that it is possible that quite special conditions were present. The only work of the above mentioned French authors to which we have had access has contained no detailed reports and no illustrations. The reader is therefore unable to gauge or form an opinion of the significance of the character and degree of the changes described. However, it may be said of the sympathetic ganglia of the intestines, as of the sympathetic ganglion cells in general, that the range of variation is great, and that it is not easy to draw the line between the normal and the pathologic. In our material also there have been found pale cells, angular cells with large indentations, and apparently large satellite cells. In certain places also, a single cell seemed to have been replaced by two or three satellite cells. But the same picture was also visible in the normal controls, so no special significance can be attached to it. The apparent disappearance of cells which are »replaced« by two or three satellite cells may well be the outcome of the section running an eccentric course; and the »pseudo-degenerative« changes mentioned above may reflect, post-mortem influences, and can not be regarded as a pathologic finding.

There is therefore every reason for being very sceptical with regard to this French report. At any rate its findings are not those usually associated with poliomyelitis. If the changes reported really existed, they must have been very special to this case. The objection may be raised to the examination in this material that two to three microscopic sections of the intestines include an exceedingly small portion of the sympathetic ganglia in them. However, if there were considerable changes in these ganglia one might expect to find such changes in at least one case.

With regard to the sympathetic ganglia of the sympathetic trunk the findings were quite negative in three of the cases in this material (table 3), and in six cases the changes were so slight as to be insignificant; they might well have had nothing to do with the poliomyelitis. In three cases the changes were more marked, and were probably due to the acute disease. In three other cases the changes

were certainly due to the poliomyelitis, but in these cases also the changes were slight. It is therefore plain that such changes are inconstant seeing that nine out of 15 cases were negative, three were doubtful, and three were definitely positive. This investigation fully supports Pette's statement, quoted above, that the sympathetic ganglia often escape changes. In those cases in which changes occur, they are slight, particularly when they are compared with those occurring in the anterior horns or the spinal medulla. But even if the comparison is made with the changes in the spinal ganglia, it will be noted that the latter are considerably more damaged in the cases examined than

Table 3.

Changes in the Sympathetic Ganglia in the Poliomyelitis Cases.

Total number	No definite changes	Small focal or perivascular infiltrations	Larger cellular infiltrations, possible neuronophagia	Severe cell change and neuronophagia
15	3	6	3	3

are the sympathetic ganglia. Only in one case were the degenerative cell changes and the neuronophagia fairly extensive (case 8). But even in this case, several cells had completely escaped damage, hardly more than 10 to 20 per cent. seeming to be involved. These findings do not suggest that the virus as a general rule follows this path to the central nervous system. If it did so, one might expect to find definite changes in the overwhelming majority of the cases, not only in a few as in my material.

Further, in the three »positive« cases, the process in the sympathetic ganglia seems to have been less advanced and therefore probably of more recent date than the changes elsewhere in the nervous system. Thus, if we consider the case in which the changes in the sympathetic system were most extensive (case 8), the neuronophagia in the spinal ganglia and in the central nervous system itself were more advanced, whereas it was in its early stage in the sympathetic ganglia (cfr. Figs. 4 and 5 with Figs. 9 and 10). A more careful scrutiny of the other cases points the same way. This seems rather to suggest that the virus as it spreads through the nervous system may also in certain cases reach the sympathetic ganglia and provoke changes in them.

It is conceivable that the tuberous swellings on the nerve fibres already referred to may be produced by the virus on its passage through the ganglia. But this conception is not plausible, firstly because this condition was also noted in the control material, in a slighter degree perhaps, secondly because it is not likely, from our general knowledge of the action of the virus of poliomyelitis, that such a change could be the immediate result of virus action. If these

changes are pathologic, it is natural to regard them as the result of damage to the ganglion cells. The perikaryon of these nerve fibres is probably situated in the spinal medulla in which case the tuberous swellings are simply due to damage by the virus which in its course has also reached these cells. Among neuropathologists local swellings of the axis cylinders are regarded as absolutely abnormal. The question whether these alterations are degenerative, regenerative or merely compensatory hypertrophic phenomena is, however, unsettled.

Thus no anatomic proof has been produced to show that the virus travels through the sympathetic nervous system on its way to the central nervous system. On the contrary, the available evidence suggests that the virus has already been present in the central nervous system when it extends to the sympathetic. It would of course be easy enough to assume that the virus travels along the sympathetic fibres without provoking in the ganglia changes which are demonstrable by the histologic methods in use, but such an assumption is not very plausible, notably because in some cases the virus can provoke changes which are characteristic and which can be revealed by ordinary histologic technique.

These results afford no final proof against the intestines as the path along which the infection travels. It is quite possible that the virus has little affinity for these ganglion cells and that as a rule it passes through the ganglia and thence in a central direction, only at a later stage and in certain severe cases following a retrograde course to the sympathetic ganglia in which it provokes changes. It would appear from the studies of *Marinesco* in 1911 that the virus seems to have little affinity for the sympathetic ganglion cells. He injected poliomyelitis virus into the ganglion cervicale superius of a monkey. The virus spread along the sympathetic fibres to the spinal cord, medulla and pons, provoking considerable changes therein, particularly on that side on which the injection had been given. The sympathetic ganglion itself showed essentially vascular changes, and only slight changes in the ganglion cells and their satellite cells.

The case in which the changes in the sympathetic ganglia were most extensive was No. 8 — a remarkably acute case which terminated fatally in less than two days. Here there were strikingly extensive changes in the whole of the nervous system, in the spinal cord, medulla and pons, the brain-stem, the cortex and the spinal ganglia. In no other case in our material was there such extensive and considerable damage. This might indicate that it is particularly such acute and severe cases in which changes are found in the sympathetic ganglia, but the other positive cases (No. 4 and No. 11) ran a course lasting six and 14 days respectively, and the changes in the nervous system were not more strikingly extensive than in several of the other cases. No definite conclusion can therefore be drawn in the sense indicated above.

It is remarkable that the French authors *Mouriquand*, *Dechaume*, *Sédaillian* and *Morin* found such extensive changes in their case. However, what has been said of the sympathetic ganglia in the intestines applies also to this case. In the one report to which we have had access, there were no illustrations or detailed description to aid the uninitiated in forming an opinion of the findings. Here, too, we must keep in mind the wide range of variation within physiologic limits; and it is certain that many pathologists do not sufficiently take into account these normal variations. Thus things definitely normal become stamped as pathologic, — a state of affairs to which we hope to return in a special study.

When a comparison is made between the changes in the peripheral sympathetic ganglia and the findings elsewhere in the nervous system, one notes in the first place that the changes are very slight and limited in extent. It is also striking that the infiltrating cells about the damaged ganglion cells are considerably less numerous in the sympathetic nervous system than in other parts in which the cell infiltration is fairly massive. This becomes plain on comparing Figs. 4 and 5 with Figs. 9 and 10. It would seem that there is quite a considerable difference in the degree of the reaction without there being any difference in its nature. It is, however, possible that this weak mode of reaction is, in a morphologic sense, a feature common to the whole of the sympathetic nervous system. Thus in his great monograph on the sympathetic nervous system published in 1924, *Laignel-Lavastine* states that the sympathetic ganglia show a much less marked morphologic reaction to injury than the spinal ganglia.

Conclusions.

1. The olfactory bulbs do not show any histologic alterations in human poliomyelitis. There is thus no histologic proof that the virus enters the central nervous system by this route.
2. The sympathetic ganglia in the intestines are without histologic changes in human poliomyelitis.
3. The peripheral sympathetic ganglia and the ganglia of the vagus nerve show small and inconstant changes in human poliomyelitis.
4. The process in the sympathetic ganglia and in the ganglia of the vagus nerve appears to be of younger date than elsewhere in the nervous system.
5. There is thus no histologic proof that the virus spreads from the intestines along the sympathetic fibres to the central nervous system.
6. These results do not conclusively rule out the intestinal tract and

the sympathetic fibres as the pathway of the poliomyelitis virus in the usual infections in human beings, but speak against this route of infection.

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ON CATALASE IN STAPHYLOCOCCUS AUREUS

By *Sven Brandt*.

(Received for publication July 8th, 1944).

It has long been known that a number of microorganisms possess the quality of splitting hydrogen peroxide into water and oxygen, and several attempts have been made (8, 11, 12, 20, 21, 22*) to find a quantitative measure for this *catalase effect* of different bacteria; but only a few under experimental conditions corresponding to our present knowledge of catalase. (See about this: 2, p. 2651 and foll., and 3, p. 1 and foll.).

Thus, *Virtanen* and co-workers (25, 26) have shown, with regard to a number of bacteria capable of splitting hydrogen peroxide, that this splitting under suitable experimental condition takes place as a monomolecular process; and they have used the activity constant k as expression for the amount of catalase present. The constant, k , is determined by titration of the fall in the hydrogen peroxide concentration during the splitting; H_2O_2 being determined with $KMnO_4$. They also found departures from the monomolecular splitting, however depending on the species of bacteria, the temperature under which the experiment was carried out and other conditions; still, these variations were so slight that they nevertheless believed they could use the ratio of k to the number of bacteria in the same volume

$\left(\frac{k}{\text{no. of bacteria per ccm.}} = \text{Kat. v} \right)$ as expression for the catalase content of the bacterial species in question. They determined this Kat. v. for a number of bacteria and showed that it is constant for the species and, within certain limits, independent of the age of the culture.

A number of the investigations accounted for in the following

*) The figures refer to the names and works of the authors in the »List of Literature» at the end of the paper.

were undertaken with the view of finding out under what conditions the splitting of the hydrogen peroxide is really monomolecular, so that k can be used as a reliable expression for the activity. The result of the experiments, which were all carried out with *staphylococcus aureus haemolyticus*, shows that this is only the case under certain conditions; since the speed at which the splitting takes place not only depends on the temperature at which the experiment is carried out, but is in various ways influenced by unknown elements in the cultures depending on the age of the latter.

Moreover, the experiments show that catalase in *staphylococcus aureus*, even at room-temperature, does not become inactivated even by rather highly concentrated hydrogen peroxide, and in this respect differs from a number of other catalases, such as blood catalase (1,9) and liver catalase (10).

Finally, some experiments show that k cannot be used as measure for the absolute amount of catalase in the bacteria, but only for the amount actively present under the experimental conditions.

Technic.

The bacterium used was a hemolysing *staphylococcus aureus* cultivated at 37° C. in veal bouillon with 1 per cent peptone and 0.5 per cent common salt, and adjusted to pH 7.8. The culture itself was used for the experiments, sometimes diluted. In some experiments, bacterial emulsions were used instead. These were made by suspending the surface growth of staph. aur. from agar sheets in sterile, distilled water. In others again, bacterium-free filtrates were used, either of cultures or of emulsions after autolysation of such for a suitable length of time at 37° C. Berkefeld filters were used. Impurities in the cultures and emulsions were ensured against by repeated cultivations on blood agar.

In a few experiments, bacteria were counted by the method indicated by Wright (27): Bacteria-emulsion (-culture) in known dilution is mixed with an equal quantity of diluted blood containing a known number of red cells. The number of bacteria in proportion to the number of these cells is determined by differential count in Leishmann-stained smears, and as the number of cells per ccm. is known, that of the bacteria can be computed. The method is rather precarious. At five counts in the same preparation, at each of which the number of bacteria per 1000 red blood cells was determined, I found mean deviations of up to 23 per cent.

The catalase activity was determined at 0°, neutral reaction, (pH 6.8) and with 0.010-n H_2O_2 . The decomposition of the hydrogen peroxide was followed iodimetrically as indicated by Stern (23), though with use of a somewhat modified technic.

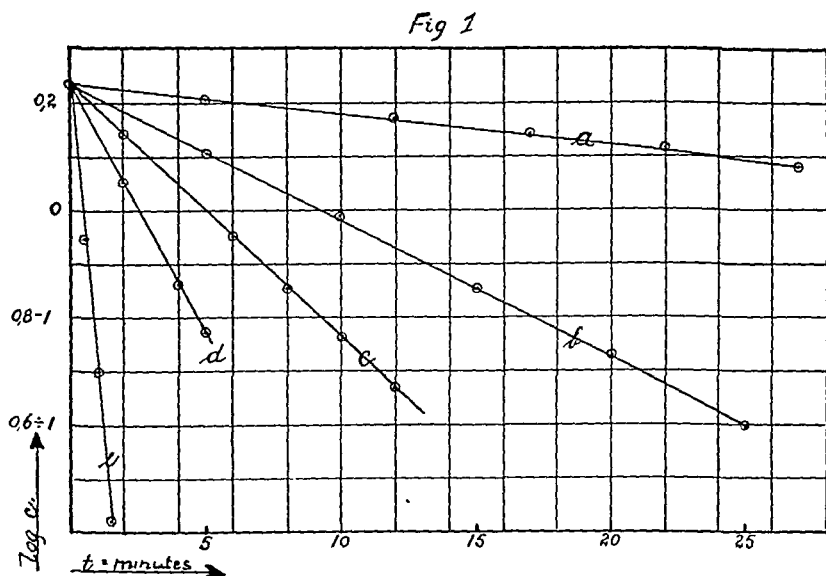
Immediately before the experiment, a 0.0176-n H_2O_2 solution was prepared by making a 1.0 per cent solution of Merck's Perhydrol (the latter containing 30 per cent of H_2O_2); and to 35 parts of this were added 15 parts of phosphate buffer, pH 6.8 (1:15-m). Of this mixture, 5 ccm. were measured out into a number of cylindric, flat-bottomed glasses (Hagedorn & Norman Jensen's blood-sugar glasses), and these were placed on ice-bath together

with the sample whose catalase activity was to be tested. When the temperature had become 0° in the glasses, 1 ccm. of the sample was, with a syringe pipette, added to the contents of each of them, at 10 seconds' intervals. The enzymic process in the glasses was interrupted at the desired moment (t) by adding, with a syringe pipette, 1.4 ccm. of 33 per cent sulphuric acid. (1.4 ccm. gives the same sulphuric acid concentration in the mixture as obtained with Stern's technic). The remaining undecomposed quantity of hydrogen peroxide was reduced by adding 1 ccm. of a 10 per cent potassium iodide solution. The oxygenation of the iodine ions was stimulated with 3 drops of saturated molybdic acid solution.

The glasses were now removed from the ice-bath and placed in a bored-out block of wood, in which they were well screened off from the daylight. (The »spontaneous« oxygenation of the iodine ions will then during the first hours be insignificant). The liberated iodine was titrated with $\text{Na}_2\text{S}_2\text{O}_3$. The amount of this consumed is an expression for the quantity of hydrogen peroxide remaining after t minutes (c_t).

The original quantity of hydrogen peroxide (c_0) was determined by blind-tests with 1 ccm. of water instead of 1 ccm. of catalase preparation. (It proved unnecessary in these tests to use culture, emulsion or filtrate in which the catalase had first been inactivated by boiling).

The values for t , $\log c_t$ and $\log c_0$ were plotted in a coordinate system, with t as abscissa and $\log c$ as ordinate. If the reaction is monomolecular, the plotted points will lie on a straight line corresponding to the equation: $\log c_t = \log c_0 \div (k \times t)$; whereupon k is calculated graphically as the inclination of the straight line (Fig. 1).



Catalase activity at different concentrations of fresh aqueous emulsions of *Staphylococcus aureus* haemolyticus from 24-hour old agar-sheet cultures. (Exp. no. 50).

	Rel. concentration	$k \times 10^4$	$k \times 10^4 / \text{Rel. conc.}$
a:	1	60	60
b:	4	253	63
c:	8	475	59
d:	16	920	58
e:	80	5410	68

As it will be seen from Fig. 1, there is proportionality between the quantity of bacteria and the catalase activity k .

Remarks on the experimental Conditions used.

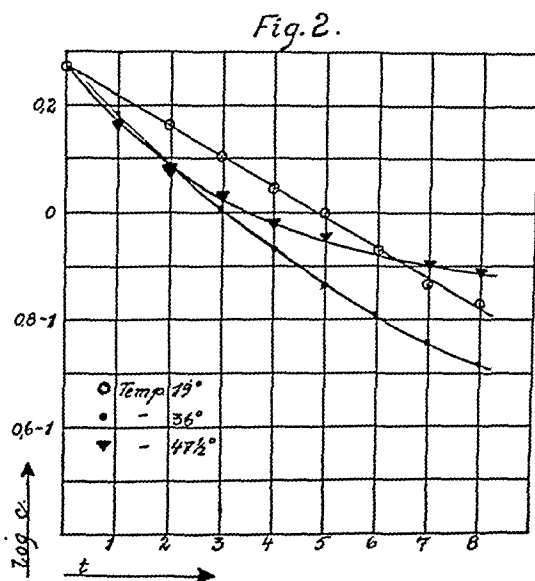
The Hydrogen Ion Concentration. — As it will be seen from Table I, the optimum pH for catalase in staphylococcus aureus is at neutrality. At reactions below $\text{pH} = 5.0$ the activity diminishes noticeably. At the alkalic reactions it does not diminish so much; the conditions are here complicated by the lability of the hydrogen peroxide in alkaline fluid.

Table I.

Emulsion of Staphylococcus Aureus. — The Dependence of the Catalase Activity on the Hydrogen Ion Concentration.
Experiment 46 d.

pH	$k \cdot 10^1$
3	70
5	272
6	294
7	291
9.2	201
11.0	207

The Significance of the Temperature for the Course of the Decomposition. — As Fig. 2 shows, the decomposition is monomolecular at an experimental temperature of 19°C ., just as at 0° . First at higher temperatures (36° and 47.5°) does the reaction deviate from the monomolecular course, and this deviation becomes more marked the higher the temperature used.

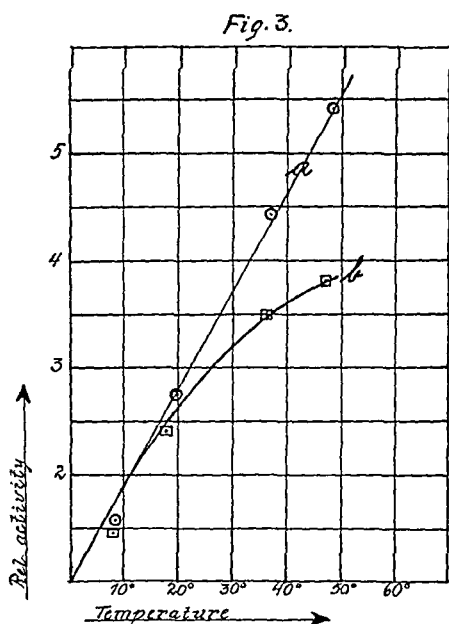


Showing the significance of the temperature for the course of the reaction.
(Exp. 53f).

A measure for the catalase activity at these temperatures can be obtained, however, by calculating k , according to the formula: $\log c_t = \log c_0 - (k \times t)$, for a number of different values of t , and then from the decreasing k -values found, determining k for zero time either graphically or by some other method (see (1)).

This deviation from the monomolecular course of the decomposition is known from other catalase studies, and is considered as an expression for the catalase's becoming inactivated by the hydrogen peroxide. For blood catalase (1, 9) and liver catalase (10) it has been found, though slightly pronounced, already at 0°C ., and has been the principal reason why the low temperature has been used in the experiments. Though the experiments mentioned above cannot be said to furnish any similar argument for working at 0°C . instead of at room temperature when the question is of staphylococcus catalase, it will nevertheless be best also here to use the low temperature, in order to avoid multiplying of the bacteria during the experimental period.

The dependance of the catalase activity on the experimental temperature will be seen from Fig. 3. The activity constants k_0 for the higher temperatures, where the reaction deviates from the monomolecular course, were determined by graphic extrapolation, as mentioned above.



Showing the significance of the temperature for the catalase activity in emulsion of staphylococcus aureus. (Exps. 53e, f, g and h).

a: Freshly prepared emulsion.

b: Emulsion kept for 3 months in ice chest.

The temperature coefficient Q_{10} (= the increase in activity per 10° rise in temperature) can be read from the ordinate. With fresh bacterial emulsion it was about 1.9. With emulsion that had been kept in ice chest for three months the coefficient was somewhat lower for temperatures between 0° at 20° C., and at higher temperatures the conditions were entirely deviating. The two experiments shown could both be reproduced.

The dependence between the relative activity ($k \times 10^4 \times S$) and the hydrogen peroxide concentration (S) with constant quantity of enzyme, differs, as will be seen from Table II, according to whether the experiment is performed with culture filtrate or with bacterial emulsion. With the former, the relative activity, as the concentration increases, reaches its limit-value more quickly than with the latter. As the Table shows, it looks as if the relative activity in experiments with filtrate even has a tendency to diminish again when the hydrogen concentration becomes sufficiently great. This makes calculation of *Michaelis's* constant difficult. It has only been possible to determine it approximatively; and with this reservation I found it to be 0.10 for culture filtrates calculated according to *Hennichs's* (10) method for determination of K_S , and about 0.12 according to *Josephson's* (13)

For bacterial emulsions it cannot be determined, but the figures

Table II.
Showing the Dependence of the Activity on the Hydrogen Peroxide Concentration.

Experiment No.	Hydrogen peroxide-molarity (S)	$k \times 10^4$	Rel. activity ($S \times k \times 10^4$)
1) Bacterial emulsion (Exp. 34)	0.0055	65	0.36
	0.011	59	0.65
	0.0225	55	1.24
	0.045	50	2.25
	0.0895	48	4.30
2) do. (Exp. 41)	0.041	201	8
	0.082	190	15.5
	0.164	187	31
	0.327	167	55
	0.654	104	68
3) Filtrate of 2 weeks' bouillon culture (Exp. 64)	0.022	123	2.7
	0.045	83	3.74
	0.065	58	3.76
	0.090	41	3.67
4) do. (Exp. 64a)	0.0055	197	1.09
	0.011	163	1.80
	0.022	139	3.13
	0.045	75.5	3.40
	0.067	50	3.35
	0.090	33.4	3.00

show that the affinity between catalase and hydrogen peroxide in these is considerably less, inasmuch as there, even at rather high concentrations of hydrogen peroxide, is very nearly proportionality between S and $(k \times 10^4 \times S)$.

Stability.

It can be shown that the power of *staphylococcus aureus* to decompose hydrogen peroxide remains unchanged for a long time when the culture is kept at about 0°C. , where no propagation of bacteria occurs. Thus, the reactivity of an emulsion kept in ice chest remained the same during 16 days. In preparations kept at higher temperatures, the catalase action at any time will be determined by (1) the propagation of the bacteria and their formation of catalase at the given temperature, (2) the temperature lability of the catalase, (3) the manner of keeping (dried, in cultures, aërobic or anaërobic), (4) the composition of the culture medium, (5) more or less uncontrollable factors, such as activation by heat (see the following) and inhibitors or activators formed during the growth and autolysis of the bacteria. In cultures left standing at 37°C. , the activity will continue to increase long after the logarithmic division phase of the bacteria. The activity in such a bouillon culture followed during fully 20 days is shown in Table III. Already after 14 hours no *measurable* increase was found in the number of bacteria. The increase that had occurred was thus compensated by autolysis of a corresponding number.

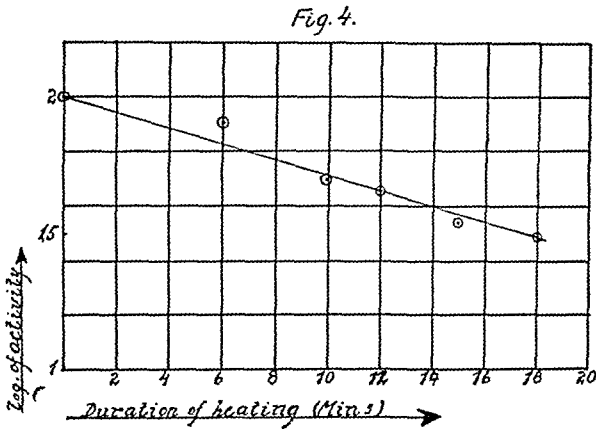
Table III.
Catalase Activity in growing Culture (Exp. 58b).

Duration of growth	$k \times 10^4$	No. of bacteria per ccm. (ad modum Wright)
14 hours	68	420×10^6
$2\frac{1}{2}$ days	320	470×10^6
$3\frac{1}{2}$ »	390	450×10^6
$6\frac{1}{2}$ »	1080	enumeration impossible owing to autolysis.
$13\frac{1}{2}$ »	2460	
$16\frac{1}{2}$ »	2730	
$20\frac{1}{2}$ »	3240	

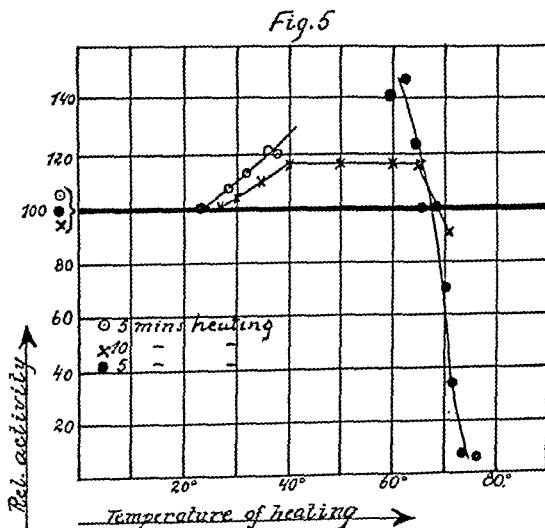
The increased catalase activity observed under such conditions may be an expression for the formation of more catalase than is destroyed per time unit at 37°C. ; but it may also be due to entirely different conditions, connected with the autolytic processes, so that the activity may not be a measure for the *quantity of catalase actually present*, but only for the *active* quantity.

Owing to the complicated conditions, such experiments do not tell anything about the stability of the catalase itself. This can better be studied in experiments of short duration, where there is no time

for propagation of bacteria to occur. Such experiments have shown (1) that the catalase becomes momentarily inactivated by boiling; (2) that it becomes inactivated with increasing rapidity at temperatures between 65° and 75° C., thus, at 75° in the course of five minutes, and that the inactivation proceeds as a monomolecular process (Fig. 4); (3) that, on the other hand, no decrease in the activity is observed even after 40 minutes' heating at 60° C.; (4) that the capacity of the bacteria for propagation is destroyed by temperatures (60° – 65° C.) at which the catalase does not become inactivated; and (5) that exposure of the bacteria for a suitable length of time to temperatures



Showing inactivation of catalase in staphylococcus aureus at 70° C. — A monomolecular process. (Exp. 56b).



Showing catalase activity after 5–10 minutes' heating of aqueous emulsions of *Staphylococcus aureus* to different temperatures. (Exps. 55c, h and p).

between 25° and 65° C. produces an increase in the activity of up to 40 per cent, which cannot be explained by the propagation of the bacteria.

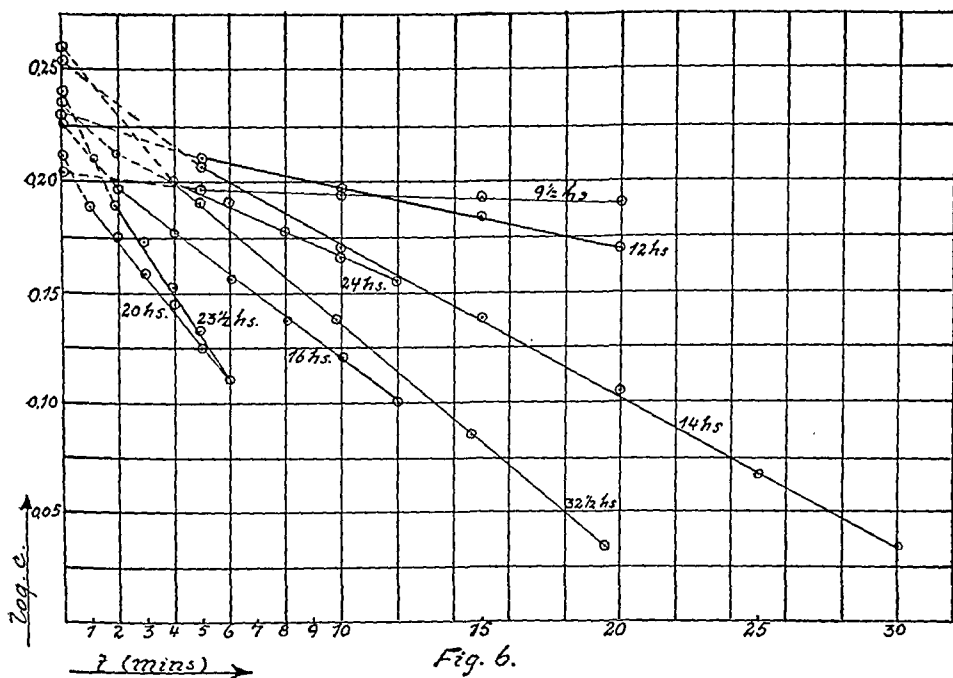
In Fig. 5, some of these observations are illustrated by three experiments. The points marked off indicate the degree of activity found in the usual manner at 0° C. after 5 (resp. 10) minutes' heating at various temperatures (the abscissa). The experiments did not show any absolute relationship between the heating time and the degree to which the activity was increased.

The durability of the catalase in the presence of hydrogen peroxide has already been spoken about (Fig. 2).

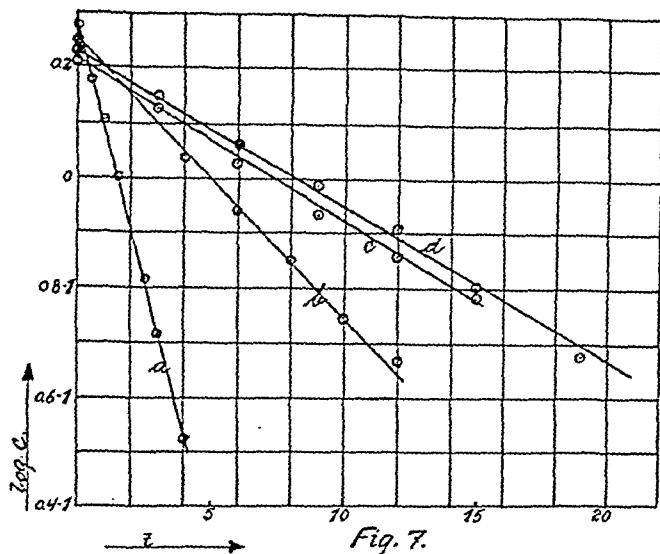
The Activity of the Catalase in Cultures of different Ages.

While the activity of the catalase in emulsions freshly prepared and kept at about 0° C. can be expressed as the constant in the formula for the monomolecular process, the same can only with a certain reservation be done as regards its activity in bouillon cultures left standing at 37° C.; because the monomolecular course of the reaction is affected and obscured in various ways, depending on the age of the culture. This will be seen from the Figs. 6, 7 and 8.

By *young* cultures are meant cultures less than 8 days old; by *older*, cultures from 8 days to about a month old; by *old* cultures, such as are over a month old.



Showing catalase activity in young cultures (Exps. 58a-b and 60). — In the beginning (the dotted portion of the curve) the reactions is *not* monomolecular.



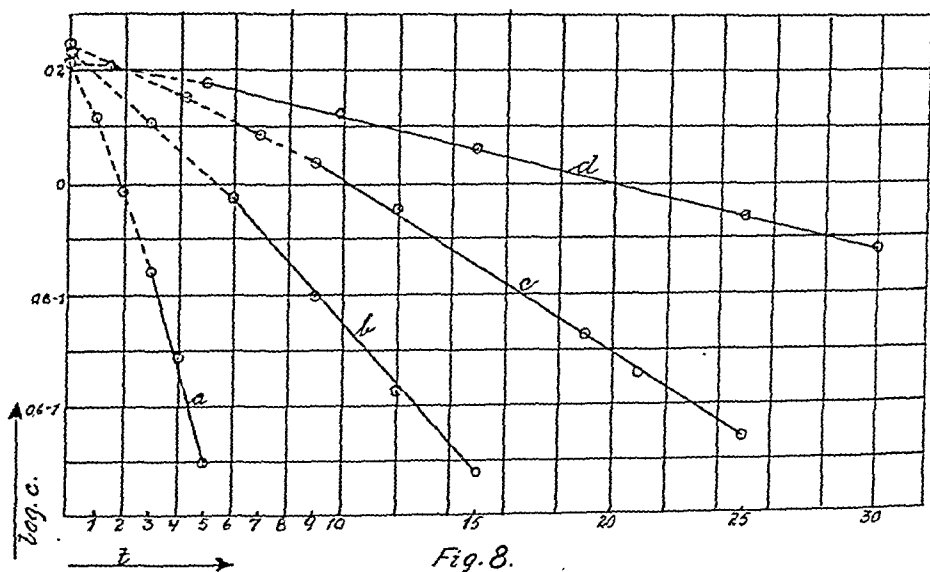
Showing the catalase activity in older cultures. (Exps. 58a and b). — The reaction runs true to the monomolecular course.

a: Bouillon cultures 14 days old.

b: " " 9 " "

c: " " 17 " " (diluted).

d: " " 17 " " (").



Showing the catalase activity in old cultures (Exps. 23f, 40, 59b and d). — In the beginning, the decomposition is inhibited; later the reaction becomes monomolecular.

a: Bouillon culture 7 weeks old.

b: " " 2 months "

c: " " 2 " "

d: Filtrate of aqueous bacterial emulsion kept for 5 days at 37° C.

In aqueous bacterial emulsions autolysis at 37° C. occurs far more rapidly, so that they already after 5—6 days behave as »old« cultures.

From Fig. 6 it will be seen that in the eight experiments with young cultures the decomposition of the hydrogen peroxide does not become monomolecular until after some time. In the beginning (the dotted part of the curve) it takes place more rapidly (? other enzymes; ? peroxidasis). This phenomenon is not seen in the older cultures (Fig. 7); there the decomposition is wholly monomolecular. If the culture is left standing for a sufficiently long time at 37° C., and the autolysis thus becomes considerable, the decomposition of the hydrogen peroxide will again deviate from the monomolecular course (Fig. 8). It will now be slower in the beginning, and will not become monomolecular until after some time.

The Activity in Filtrates.

It has been discussed whether bacterial catalase should be considered as an endoenzyme or an ectoenzyme; some investigators (12, 13) having found catalase activity in filtrates of cultures, others not. My own experiments with staphylococcus aureus have shown that filtrates of young cultures are quite inactive. On the other hand, active filtrates could be produced by letting the cultures or the aqueous emulsions undergo autolysis for a suitable length of time at 37° C. (Porcelain filters should be used, as Zeiss-filters absorb the catalase). Aqueous emulsions autolyse rapidly, so that activity in the filtrate can be demonstrated already after a few days (on the other hand it is very difficult to avoid pollution by other bacteria). In cultures, the autolysis mostly requires a little longer time.

Owing to the conditions mentioned in the foregoing (Fig. 8), it is difficult to estimate the degree of the activity in filtrates of auto-

Table IV.

Relative increase of Activity in diluted Filtrates of autolysated bacterial Emulsions.

	Concentration (=enzyme quantity)	$k \times 10^4$	Remarks
1) Exp. 47b	undiluted 1:10	735 96	Course of the decomposition: cf. Fig. 7.
2) Exp. 47c	undiluted 1:10	2225 550	Course of the decomposition: cf. Fig. 8.
3) Exp. 23f	5:50 4:50 3:50 2:50 1:50	151 125 92 52 32	} Course of the decomposition: cf. Fig. 8. } Decomposition monomolecular.

lysed cultures; still it may probably be permissible to calculate k on the basis of the rectilinear part of the curve. But to this comes the other circumstance that it, as will be seen from Table 4, only in highly diluted filtrates has been possible to demonstrate the same proportionality between enzyme quantity and k as in the case of freshly prepared emulsions (cf. Fig. 1); irrespectively of whether the decomposition in the filtrate follows the monomolecular course or deviates from it in the manner described.

Discussion.

In several respects, the experiments confirm the results of earlier catalase studies; thus, as regards the proportionality between enzyme quantity and activity found in emulsions, cf. Fig. 1 (1, 2, 3, 9, 10 and others), a proportionality found also with catalase from yeast and from other bacteria (8, 11, 18).

On the other hand, the experiments have shown that a similar proportionality cannot be counted upon when the catalase occurs extracellularly in autolysed cultures or filtrates of such; the activity by dilution becoming greater than corresponding to the degree of the latter (Table IV). Only in the high dilutions do we again find proportionality. It thus seems that the autolysis has resulted in the formation of inhibitory substances, the effect of which can be reduced by dilution.

The experiments also seem to indicate that the autolysis may result in the formation of other inhibitory substances, which differ, however, from those just mentioned by being labile (? to the hydrogen peroxide), so that the inhibitory effect is lost when the decomposition has proceeded for some minutes; cf. Fig. 8; (compare the inhibitory effect of cyanhydrogen on catalase (6)). The assumption that these substances are inactivated by hydrogen peroxide is supported by the results of a couple of experiments not included in this present paper, in which a filtrate was diluted with a weak hydrogen peroxide solution some time before the experiment was begun. The inhibitory effect was then less than in parallel experiments in which the filtrate had been diluted with water.

The optimum hydrogen ion concentration found for the staphylococcus catalase at neutrality corresponds to what has been found for other catalases (2, 3, 15, 18, 23, 24), also for catalase from bacteria (7, 22). On the other hand, the temperature coefficient found ($= 1.9$) is a little higher than usually stated (about 1.5; see 3, p. 45). For catalase from bacteria there are no earlier statements regarding this.

The experiments have given interesting information about the inactivation of the staphylococcus catalase in the presence of hydrogen peroxide. As already said, a similar lability in the presence of H_2O_2 ,

resulting in characteristic deviation from the monomolecular course of the decomposition, is shown also by a number of other catalase preparations, among others by blood- and liver-catalase (1, 2, 3, 9, 10, 15). It is proved to depend on the temperature, the hydrogen ion concentration and the concentration of the hydrogen peroxide. With staphylococcus catalase the decomposition is, however, monomolecular even at room temperature (Fig. 2); in contrast to what is the case with catalase from blood and liver, which already at 0° C. is inactivated by hydrogen peroxide in the low concentrations generally used in the experiments. This characteristic stability of the staphylococcus catalase is further confirmed by experiments in which concentrations of hydrogen peroxide as high as n-0.18 (filtrates) and even n-1.3 (emulsions) were used (Table II). In these experiments it was not possible, in spite of the high concentrations of the hydrogen peroxide, to observe any inactivation of the catalase; the decomposition following the monomolecular course.

Experiments showing the *dependence of the catalase activity on the concentration of the substrate* (which is usually expressed by Michaelis's constant) seem to indicate that the affinity between enzyme and hydrogen peroxide is considerably less in bacterial cultures and emulsions than in filtrates, where the catalase, just as the H_2O_2 , is evenly distributed through the mixture in which the reaction takes place. Even under these conditions it is less than in experiments made with concentrated and purified catalase preparations from liver. The constant for the latter is stated by *Euler* (6) as 0.025, by *Henrichs* (10) as 0.0456. (Table II). A discussion of the cause of this lies outside the scope of this paper; but it may be pointed out that these experiments have shown characteristic differences between the determinations of catalase activity made on cellular suspensions and those made on filtrates. The question presents itself, whether it is possible, in fact, to draw quantitative conclusions from the measured activity, k , to the amount of catalase in the cell (cf. 25, 26). The experiments mentioned, which showed increase of the activity after heating of the bacteria, proved that the activity can be increased without this giving ground for believing that also the absolute amount of catalase in the cell has been increased; (by counting the bacteria before and after the heating it could be shown that the increased activity was not due to increase in the number of these). A similar activation by heat has been observed with regard to blood corpuscles (5, 16) and yeast cells (4). In the latter, an increase to twenty-five times the former degree of activity has been found after heating for 1 to 2 hours at 55 to 63° C. In most cases it is, however, a question of increase of less than 100 per cent.

Also in other ways has it been possible to increase the catalase activity in cell suspensions. Thus, a bacteriolysis (14, 17, 19) is followed by rather considerable increase in activity. *Nosaka* (16) thinks

that the increase in the activity of blood corpuscles after heating is due to hemolysis. That a corresponding bacteriolysis has not occurred in my experiments is proved, however, by the fact that no activity could be demonstrated in filtrates of bacterial suspensions that had been subjected to heating.

Euler has found increase of the activity not only after heating, but also after treatment of yeast cells with chloroform or toluol; and from his observations he draws the conclusions (3, part I, p. 268) that »Die Katalasewirkung von Zellen wird durch Faktoren beeinflusst, welche wir noch nicht beherrschen. Wir können also von der Katalasewirkung noch nicht auf die Katalasegehalt schliessen«.

It is also rather difficult to imagine that the considerable increase of activity in growing cultures after the logarithmic division phase should be due solely to continued catalase production in the form of cell multiplication.

Summary.

The author has studied the catalase effect of *staphylococcus aureus haemolyticus* on hydrogen peroxide under various conditions; the decomposition of the H_2O_2 being followed iodimetrically. It is shown that

1) the reaction is monomolecular in resting, not autolysed, aqueous emulsions and in cultures of suitable age;

2) the optimum hydrogen ion concentration is about at neutrality;

3) the inactivating effect of the hydrogen peroxide on the catalase is not observed until at temperature between 30 and 40° C.; in which staphylococcus catalase differs from a number of other catalases, which become inactivated already at 0° C.;

4) Q_{10} (for temperatures between 0 and 20° C.) lies at 1.9 or somewhat lower;

5) the affinity between enzyme and H_2O_2 is considerably less in emulsions than in filtrates containing catalase, and Michaelis's constant for the latter is approximatively 0.10;

6) by heating above 65° C., the catalase becomes inactivated with increasing rapidity, at 75° C. in the course of 5 minutes; and the inactivation is a monomolecular process;

7) heating at temperatures between 25 and 65° C. increases the activity;

8) with young, rapidly growing cultures the decomposition of the hydrogen peroxide is in the beginning more rapid than with older ones; presumably owing to the presence of other enzymes, whose action soon ceases;

9) cultures in which the autolysis is pronounced contain inhibitory substances whose effect is diminished by dilution, and other inhibi-

tory substances whose action becomes less as the decomposition proceeds;

10) activity in filtrates can be demonstrated after autolysis has been going on for a suitable time.

It must be doubted if the activity-constant found in emulsions and cultures is a quantitative measure for the *absolut* amount of catalase in the bacteria, even when it is ensured that the conditions under which the experiments are carried out are such that the decomposition will follow a monomolecular course. The activity constant can only be a measure for the *active* amount of catalase in the cells.

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STUDIES ON SERUM PHOSPHATASES AND SERUM PHOSPHORUS IN NORMAL RABBITS, BESIDES THE INFLUENCE OF STARVATION ON THESE VALUES

By *Gunnar Jorgensen.*

(Received for publication July 20th 1944).

Only few studies have been reported on serum phosphatases in normal individuals. In Denmark a fairly comprehensive work on this subject has been published by Vermehren who carried out his studies on children and adults. In this investigation the diurnal variations in the plasma phosphatase content in normal infants were found to be very slight, and a 24-hour water diet and ingestion of 1.5—2 g. glucose per kg of body weight did not affect the phosphatase content. The values for plasma phosphatases were found to be increased in pregnancy and lactation. Normal infants (0—2 years) showed seasonal variations in the plasma phosphatase content, with higher values in the winter than in the summer.

Further, mention is to be made of the papers published by Bodansky & Jaffe, showing that the plasma phosphatase content in young animals is particularly sensitive to dietary changes. Experiments carried out on rats as well as dogs showed that undernourished and starving animals give lower phosphatase values than do well-fed animals. Finally, digestion of carbohydrates was found to give higher plasma phosphatase values whereas digestion of meat gave lower values.

Various reports by Bodansky & Jaffe, Oluf Andersen, Stearns & Warweg show that the phosphatase values are higher in adolescence than in adult persons.

The findings here mentioned are associated with the peculiar conditions connected with the presence of the phosphatases in the blood. Generally the phosphatases in the blood are reckoned to represent a sort of waste products. In the various physiological or pathological processes taking place in the various organs in which the phosphatases play a certain part, these substances are given off to the blood stream without serving any purpose in the blood. It is

obvious, then, that variations in the serum or plasma phosphatases in normal animals signify that various »phosphatase processes« take place with greater or lesser intensity in some of the organs.

A-priori, then, one would expect the phosphatase activity in the serum to be subject to very great variations. Nevertheless, as demonstrated by Vermehren, the phosphatase level is found to keep practically at a constant level; and this must mean that the processes here involved practically go on fairly continuously. This concerns many different processes, of which the more important are associated with the digestion, resorption of glucose from the tubules of the kidney and the building up of bone. But, it cannot be taken for granted that the same conditions prevail in other animal species. With a view to this point, it is of particular importance to ascertain the

Table 1.

Phosphorus and Phosphatase Determinations on Normal Rabbits.

P = serum phosphorus in mg.%. F = serum phosphatases per 100 cc. of serum.

Time	0		$\frac{1}{4}$ — $\frac{1}{2}$ hour		1 hour		4 hours		24 hours	
No.	P	F	P	F	P	F	P	F	P	F
1	4.04	4.78								
2	3.84	5.51								
3	5.12	5.07								
4	4.68	6.77								
5	3.88	3.37					3.80	3.36		
6	4.31	5.04								
7	3.48	7.17			3.31	7.34			3.56	5.57
8	4.36	5.62								
9	4.64	4.81								
10	4.80	7.59	4.84	7.66					3.36	5.36
11	3.52	3.60								
12	3.28	4.72								
13	3.88	3.57			3.84	3.51			4.16	3.71
14	4.40	4.31								
15	4.60	4.85								
16	4.84	7.55								
17	3.52	6.35								
18	3.76	4.85								
19	3.52	5.19	3.56	5.47					4.12	4.49
20	4.12	4.81								
21	4.24	6.47								
22	4.36	7.40	4.36	7.50					4.40	6.52
23	4.24	8.36								
24	4.16	7.18								
25	3.88	4.00								
26	3.99	6.51								

character of these conditions in the rabbit, because the rabbit is an inexpensive animal and easy to manage.

There are two things that are important to know: the range of variations in normal animals, and whether the phosphatase in serum keeps at a fairly normal value within each individual animal. In order to answer these questions, the following experiment was undertaken: Table 1 gives a number of phosphatase determinations on 26 normal rabbits. The determinations were carried out with the Bodansky technique. The values obtained were found to be rather varying with 5.67 units per 100 cc. of serum, giving an average of 8.36 units as the greatest and 3.37 units as the smallest value. Freeman & Farmer, employing the same technique in the experiments on adult animals obtained an average value of 4.14 units, with 5.23 units as the maximal value and 2.37 as the minimal value. The difference between the two series of experiments is probably due to the circumstance that the animals here employed were very young, whereas Freeman & Farmer used adult animals. Accordingly it seems reasonable to reckon with

Table 2.
*Experiments on Diurnal Variations in Serum Phosphorus and
Serum Phosphatases.*

P = serum phosphorus in mg.%. F = serum phosphatases per 100 cc.
of serum.

No.			Ordinary diet						Starvation			
	1' day		5' day		7' days		9' days		1' day		2' day	
	P	F	P	F	P	F	P	F	P	F	P	F
30	3.68	4.72	4.12	2.60	4.12	3.34	3.92	3.22	3.36	2.74	3.52	1.94
27	2.96	4.92	4.08	3.75	3.64	4.24	3.56	3.79	3.60	3.85	3.56	2.01
28	3.68	5.35	3.68	5.14	3.72	5.63	3.92	6.05	3.36	4.73	3.32	3.19
29	3.20	2.21	3.40	1.96	3.52	3.73	3.68	3.04	3.40	3.11	3.28	1.97

a rather wide range of variations in the phosphatase determination of the rabbits.

The next point to investigate was whether the phosphatase value kept constant within the individual animal. For this purpose, in the same experimental series, 6 of the animals were picked out for new determinations, partly very brief initial tests, undertaken shortly after (up to 4 hours), partly 24 hours later.

In this way the variations within the first 4 hours are found to be quite small, whereas the variations in the 24-hour period are rather considerable. The fall in the values might perhaps have been due to the scanty feed given to the animals in this period. Still, one animal, No. 13, keeps constant throughout the experimental period, and it showed a low initial value (3.57 units).

Table 2 shows also a further investigation of the diurnal variation. This experiment comprises 4 animals, and the experimental period extends over 9 days. Samples of blood were taken on the 1', 5', 7' and 9' day. The experiments shows that the phosphatase content of the serum is subject to considerable variation. Thus it may be recorded that the maximal and minimal values for the 4 animals employed in this experiment were: No. 30: 4.72 and 2.60; No. 27: 4.92 and 3.75; No. 28: 6.05 and 5.14; and No. 29: 3.73 and 1.96.

Further, the variations turn out not to be uniform in the four animals, so that it would not be reasonable to imagine a certain external factor as the cause of the above-mentioned changes.

In these experiments, we found that the phosphatase values are not constant in the animals here employed. According, to the observations previously reported it seems justifiable to assume that this variability is due to various physiological processes, in which the phosphatases play an important part. In this connection, naturally, one will think of the digestion. So there is every reason to investigate whether the phosphatase values may become constant by starvation of the animals.

As recorded in Table 2, therefore, the experiment was continued with starvation of the animals. As demonstrated by Jaffe & Bodansky the starvation period was associated with a fall in the phosphatase values; and this fall was particularly pronounced after the second day of starvation, at which point of time it further was noticed that the variation between the animals had become very slight.

Simultaneously with the above-mentioned phosphatase determinations, the serum phosphorus concentration was determined too. Also these determinations were carried out with Bodansky's technique. The aim of these determinations was to ascertain the variations that may occur among a number of animals, and also to see whether the value obtained for the individual animal is constant or perhaps varying. As recorded in Table 1, determinations of the serum phosphorus were carried out on 26 normal animals. The values varied between 5.12 mg.% and 3.28 mg.%, with an average of 4.14 mg.%. Corresponding analyses carried out by Freeman & Farmer gave the following values; maximum 6.94 mg.%, minimum 3.37 mg.% average 5.04 mg.%. These values are somewhat higher and show a wider dispersion than the ones obtained in the present studies, but it is not possible to give any reason for this. So there must be said to be a rather wide range of variation.

In order to see whether the values obtained in the individual animals might be constant, the values were further examined in 6 animals of this group, within a relatively short period (4 hours) and a longer period (24 hours) after the first sampling of the blood. These determinations show that the values are practically constant within the first four hours, whereas they show rather wide variations after

24 hours, increasing as well as decreasing. The greatest variation was found in animal No. 10, in which the phosphorus value fell from 4.80 to 3.36, *i. e.*, a difference amounting to 30 %.

Some rabbits kept on a uniform diet under uniform external conditions showed rather considerable diurnal variations in the serum phosphorus concentration (Table 2). The experimental period was 9 days, and samples of blood were taken on the 1', 5', 7' and 9' day. The highest and lowest values obtained in the individual animals were as follows: No. 30, 3.68 and 4.12; No. 27, 2.96 and 4.08; No. 28, 3.68 and 3.92; No. 29, 3.20 and 3.68. This means that the serum phosphorus values under normal conditions are not constant in the individual animals but subject to variations just like the phosphatase. The same examination carried out on starving rabbits (Table 2) shows that the serum phosphorus values during inanition keep more constant, the highest variations in two days of starvation amounting to only 5 % of the initial value.

As is evident from the findings reported here, the serum phosphorus and phosphatase values in rabbits are not constant under ordinary conditions. Hence studies on these may be carried out only on taking particular measures. If we imagine a certain experimental plan, after which the experiment lasts about 24 hours, we have to demand that the phosphorus and phosphatase values keep constant within this period or possibly undergo some change in a regular manner. In addition, it would be desirable to reduce the variations between the animals as far as possible. According to the experiments here reported, this may possibly be obtained by starving the animals before the experimental period commences and keep them starving or give them but a very small amount of food during the experimental period itself.

This possibility is established through the findings recorded in Fig. 1, presenting 25 animals that were starved for 24 hours prior to the experiment. During the experimental period which lasted up to 48 hours, the animals got but little to eat — and only beets. 4 samples of blood were taken during this experimental period and the variations are here recorded graphically in such a way that the initial value is set at 100 and the variations are then calculated accordingly, *i. e.*, in percentage. The figures along the axis of abscissas give the hours after the first sample of blood was taken, and the figures on the ordinates give the percental variations.

As to the phosphatases, it will be noticed that within the first 30 hours there are variations between 106 and 89. Half an hour and 1 hour after the first sampling of the blood the variations are a little smaller, but they become as great as mentioned already 5 hours after the first sample of blood. As the mean error on the determinations has to be reckoned as being from 2 to 5%, these variations are of a somewhat greater magnitude and undoubtedly due to the fact that

the phosphatase values under the given experimental conditions are inclined to fall off somewhat — which is confirmed by the fact that the values obtained 48 hours after the first sampling of the blood lie 20—25 % under the initial values.

As to the serum phosphorus, the variations are found to lie between 105 and 94. Variations of this magnitude are found as early as 1 hour

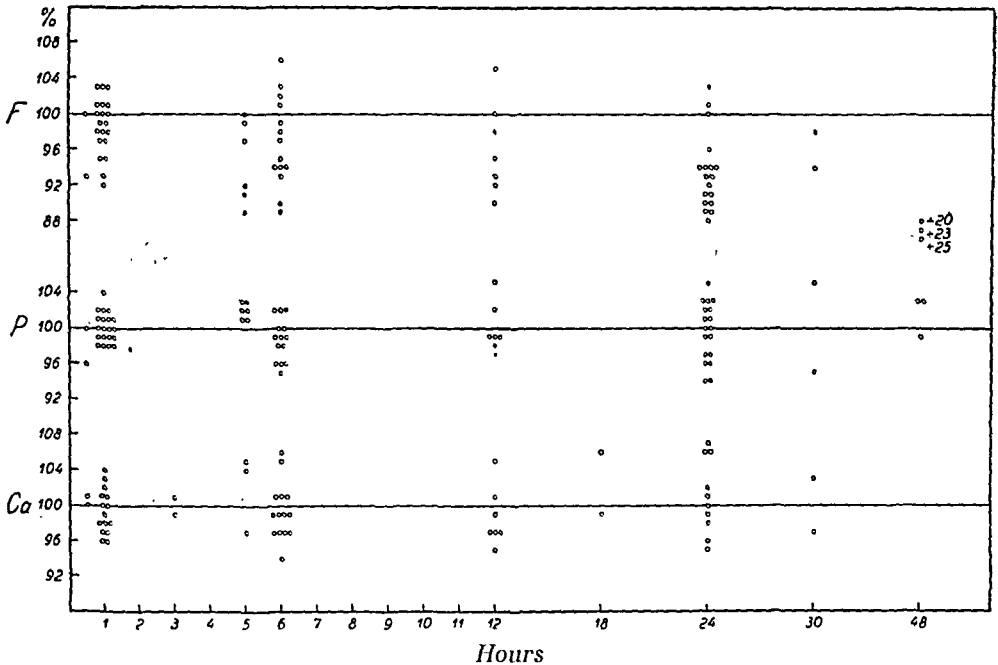


Fig. 1.

Spontaneous variations in 25 animals which were starved for 24 hours prior to the experiment and then given a scanty feed of beets. — The length of time is plotted along the axis of abscissas after the first sample of blood was taken. The percental values obtained were plotted as ordinates.

F = serum phosphatase. P = serum phosphatase. Ca = serum calcium.

after the first sample of blood, although the figures here are more uniform than 6, 12 and 24 hours after. As the mean error for these determinations is 2 %, the variations are really a little greater, which means that under the given conditions there are certain, although minor, spontaneous variations.

So, under the given experimental conditions, the phosphatase values as well as the phosphorus values keep fairly constant. In order to investigate whether the other requirement — that the range of variation must be as small as possible — is met too, Fig. 2 gives a comparison between the values for serum phosphatases and serum phosphorus obtained in two groups of animals, namely: the 26 animals mentioned in Table 1 that were not submitted to any treatment, and

68 animals treated after the above-mentioned method with starvation for 24 hours and then a scanty feed of beets.

The 68 animals gave the following values:

1) Phosphatases, average 3.38 units per 100 cc. of serum; maximum 5.18 units; minimum 1.86 units.

2) Phosphorus, average 3.83 mg.%; maximum 4.88 mg.%; minimum 2.76 mg.%.

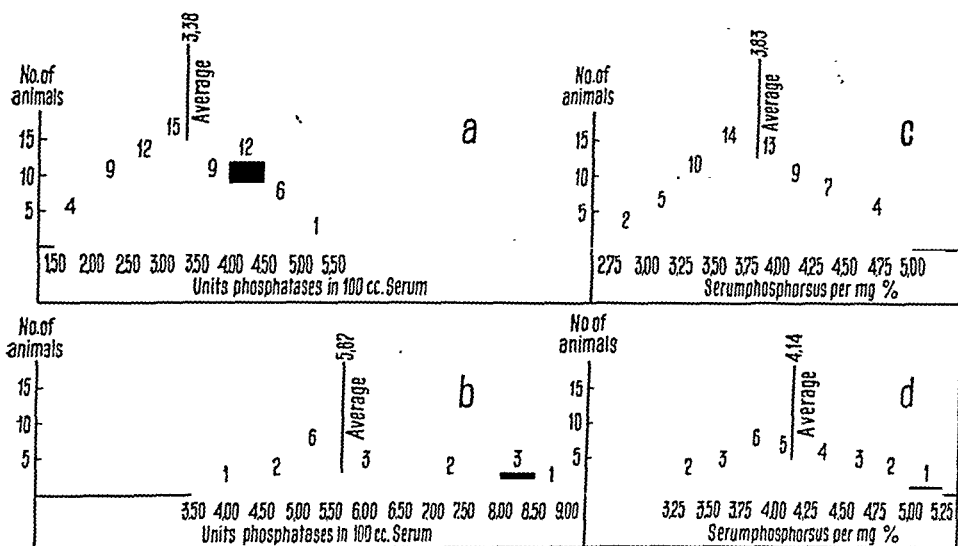


Fig. 2.

Comparison of the range of variations for serum phosphatases and serum phosphorus in 26 untreated animals and 68 animals which had been starved for 24 hours and were then given a scanty feed of beets. — The ordinates give the number of animals, while the abscissa gives the serum phosphatases in units per 100 cc. of serum and the serum phosphorus in mg.%.

- a: phosphatase determinations on 68 animals
 b: " " " 26 "
 c: phosphorus " " 68 "
 d: " " " 26 "

The averages for the various groups are indicated too.

It is plainly evident that the values obtained in these animals — for serum phosphorus as well as phosphatases — on the whole lie at a lower level than the values obtained in the untreated animals. This confirms the aforementioned experimental finding reported by Jaffe & Bodansky — that starvation lowers the serum phosphatase values. It is reasonable to emphasize this finding as the experimental material here is fairly large. The average value falls from 5.67 units to 3.38 units per 100 cc. of serum. The fall of the phosphorus values is of lesser magnitude, the average falling from 4.14 mg.% to 3.83 mg.%. Further, it is found that the range of variation for the phos-

phatases in this group of animals is considerably small, whereas the range of variation of the serum phosphorus is practically the same in the two groups.

Summary.

The serum phosphatases and serum phosphorus are demonstrated in rabbits, not to keep at constant levels but show rather wide spontaneous variations.

In 26 normal animals the serum phosphatases were seen to vary between 8.36 units per 100 cc. and 3.37 units with an average of 5.67 units per 100 cc. Serum phosphorus varies between 5.12 mg.% and 3.28 mg.%, with an average of 4.14 mg.%.

It is further demonstrated that starvation lowers the serum phosphatase values considerably and serum phosphorus somewhat.

Finally, it is demonstrated that by starving the animals for 24 hours and then keeping them on a scanty diet of beets, it is practicable to get the phosphatase as well as the phosphorus values to keep fairly constant.

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STUDIES ON THE EFFECT OF PARATHYROID INJECTIONS ON NEPHRECTOMIZED RABBITS

By *Gunnar Jørgensen*.

(Received for publication July 20th 1944).

In 1929 Albright and collaborators found the parathyroid hormone to have a distinct effect on the phosphate output through the kidneys, as this was greatly increased. Subsequent experiments by Albright, Ellsworth & Howard have further elaborated this observation so that now it can be said that the first effect of a parathyroid injection is an increase in the phosphate output that reaches its maximum in $\frac{1}{2}$ —3 hours after the injection. This increased output results in a decrease in serum phosphorus followed by a rise in serum calcium. On this account the authors advanced the theory that the parathyroid hormone exerts its primary effect on the phosphorus metabolism, and that the effect on the calcium metabolism is secondary. This view has been criticized by other authors, as presumably the processes actually taking place are not so easily surveyable as assumed by Albright and collaborators. In order to simplify the conditions, experimental studies have been carried out on nephrectomized animals. For such animals were supposed to present the uncomplicated effect of parathyroid on the blood, without this effect being concealed or masked by the calcium and phosphate output; and if the theory of Albright and collaborators were correct, it would be expected that the parathyroid hormone would be ineffective as the eliciting factor in the hormonal effect — namely, the phosphate excretion — was abolished.

Of previous studies in this direction, two experimental series have been published by Tweedy, Templeton & MacJunkin. These authors worked with nephrectomized dogs and found that even very large doses of parathyroid hormone had no effect on the serum calcium concentration. Animals with bilateral ligature of the ureters behaved like the nephrectomized animals. On one of these animals the ureteral ligature was loosened, and a new injection of parathyroid hormone

gave a marked rise in serum calcium. On autopsy neither the macroscopic nor the microscopic examination of the organs showed any signs of parathyroid effect.

In contrast to these findings, Ellsworth & Fletcher have reported some experiments on 3 dogs, in which they found a distinct rise in serum calcium, while the controls showed only a slight rise. Also the serum phosphorus rose after the injection.

Collip, Pugsley, Selye & Thomson found that in nephrectomized rats the injection of parathyroid hormone gave a distinct effect on the bones.

Thus there is a considerable divergence between the studies presented on this question. Possibly this divergence may be explained to some extent by the circumstance that dogs presumably are not very suitable animals for such experiments. In the first paper by Tweedy *et al.* the controls showed a marked rise in serum phosphorus within 24 hours after the operation; and this may be taken to signify that they became uremic rapidly. On this point, then, rabbits prove to be better experimental animals as they remain unaffected by the operation for a considerable length of time. As a rule these animals were found to be unaffected even for 48 hours, and there was no particular increase in the phosphorus content at all.

The present experimental series comprises 7 controls (Table 1). Of these animals, two (Nos. 74 and 78) were clinically affected at the commencement of the experiment, and they were getting worse during the course of the experiment. No. 77 was almost moribund at the conclusion of the experiment. These two animals showed a marked increase in serum phosphorus; but in No. 74 the calcium values were normal, without any pronounced variations. Such animals are reckoned as unsuitable for experiments of this kind and have not been employed. The other controls showed a fairly uniform fall in serum phosphorus and serum calcium. The fall amounts, at the most, to about 15 % of the phosphorus and about 10 % for the calcium. Possibly this change is attributable to the loss of blood at the operation and sampling of the blood. No. 79, which showed a very pronounced fall, was subject to an accident at the operation, as the ligature of the renal vessels on one side slipped, and this was followed by a considerable hemorrhage. In view of this accident, we naturally have tried to make the operation as bloodless as possible.

In some preliminary experiments the animals were found to be somewhat shocked for some time after the operation: for instance, it was rather difficult to obtain samples of blood. On this account we went on to operate the animals in the afternoon — before the experiment commenced next morning.

Some of the 9 experimental animals were given injections of 25 units (Nos. 81 and 82) while the remainder were given 50 units of

Table I.
Experiments on Nephrectomized Animals.

No.	Ca	P	Ca	P	Hours after inj.	Ca	P	Hours after inj.	Ca	P	Hours after inj.	Remarks
74 Control	15.80	4.56	16.20	4.32	3	16.20	4.68	6	15.20	5.72	17	Looking poorly.
75 "	15.12	5.04	15.12	5.12	2	15.24	5.12	5	14.40	4.44	17	
76 "	13.80	5.48	13.68	5.52	1	13.68	5.44	6	13.32	5.12	10	
77 "		5.60		5.04	1					8.36	24	Looking very poorly.
78 "	12.36	5.84	12.24	5.76	1	12.84	5.68	4	12.24	5.44	17	
79 "	16.68	3.12	15.84	2.92	2	15.36	2.72	7	15.12	2.68	12	Considerable hemorrhage at the operation.
104 "	12.36	6.00	12.36	6.12	2	12.72	5.96	6	12.12	5.84	8	
80 50 units	16.92	5.24	16.44	6.20	1	16.80	6.24	4	15.24	5.04	14	
81 25 units	15.60	0.00	15.72	6.72	1	16.92	6.68	4	14.52	6.00	12	
82 "	14.88	7.48	14.64	7.68	1	15.12	7.44	4	14.64	7.32	8	
83 50 units	12.84	4.08	13.56	4.28	1	13.68	4.12	6		3.68	10	
84 "	12.36	5.12	13.92	5.32	1	14.28	5.44	6	13.32	4.60	10	
85 "	15.36	2.88	15.24	3.12	2	16.56	2.84	6	15.84	2.68	11	
86 "	15.36	4.64	15.60	5.28	2	16.44	4.88	6	16.20	4.24	11	
105 "	11.64	3.12	12.72	3.52	1	13.56	3.36	6	12.96	3.20	8	
106 "	12.12	4.36	12.48	4.68	1	12.24	4.48	6	12.00	4.28	8	

Ca = serum calcium in mg. %.

P = serum phosphorus in mg. %.

25 units = 25 units of parathyroid hormone injected at the commencement of the experiment.

50 units = 50 units of parathyroid hormone injected at the commencement of the experiment.

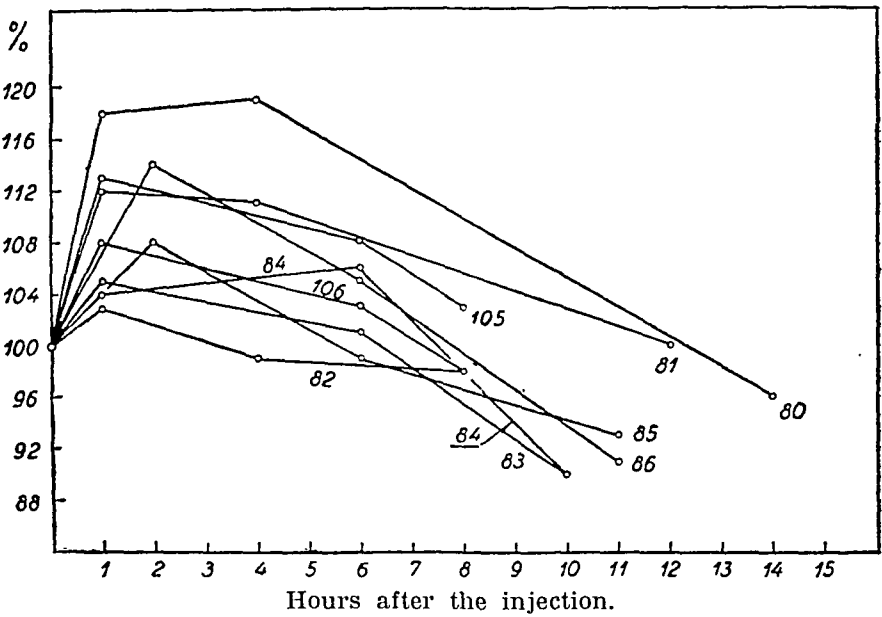


Fig. 1.

Serum phosphorus determinations on nephrectomized animals injected with parathyroid hormone.

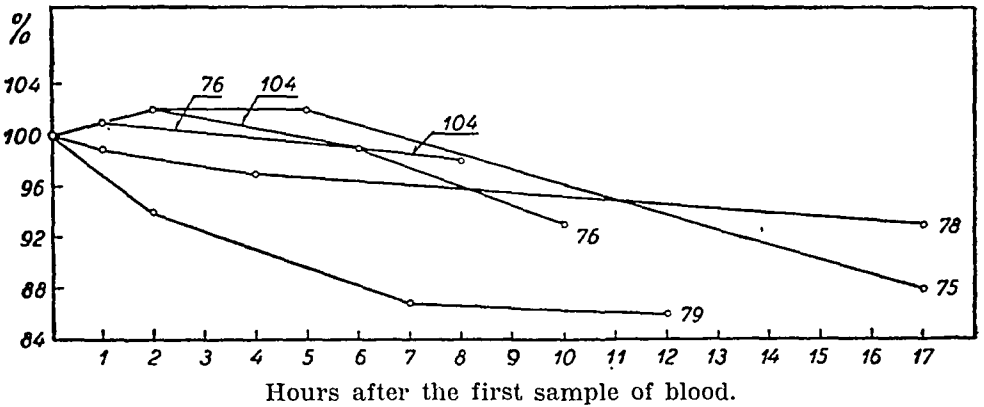


Fig. 2.

Serum phosphorus determinations on nephrectomized controls.

parathyroid hormone (Nos. 80, 83, 84, 85, 86, 105 and 106). The phosphorus determinations were carried out after Bodansky's method, the calcium determination after Brigg's method, modified by Roe & Kahn.

The values obtained are recorded in Table 1; and the results are presented graphically in Figs. 1—4, the percental values being recorded as ordinates while the time is plotted along the axis of abscissas.

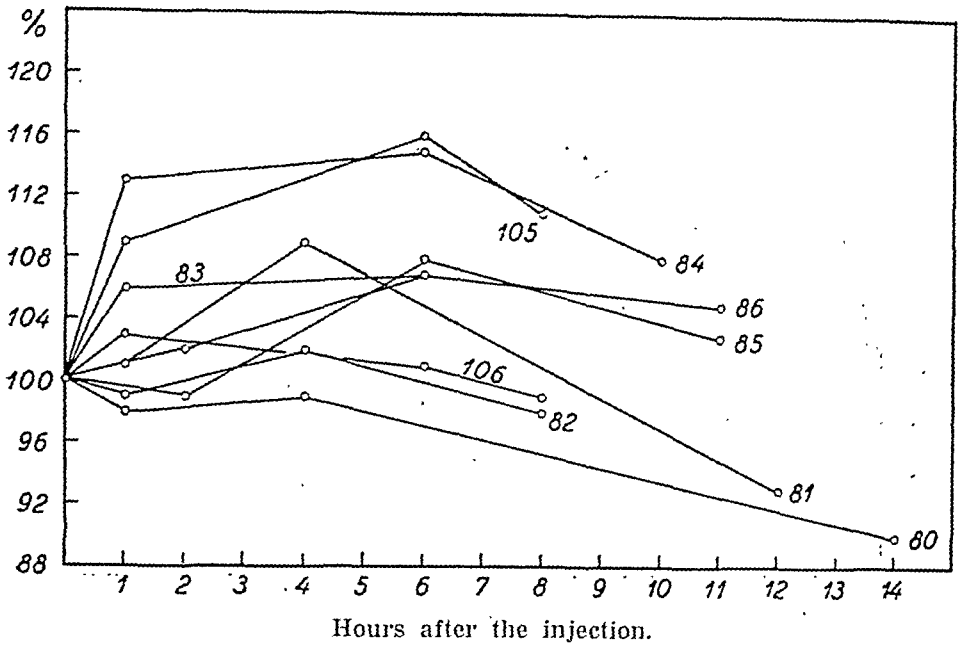


Fig. 3.

Serum calcium determinations on nephrectomized animals injected with parathyroid hormone.

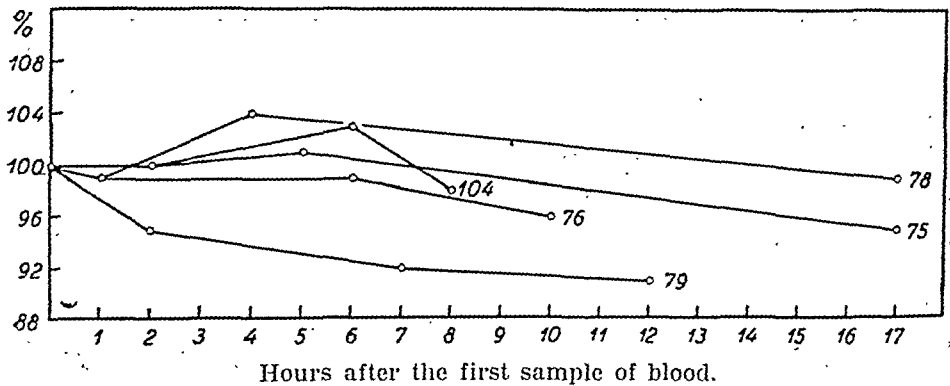


Fig. 4.

Serum calcium determinations on nephrectomized controls.

As will be noticed, there is a distinct increase in the serum phosphorus after the injection, the greatest amounting to 19% of the initial value. The height of the curve was reached shortly after the injection, 2—4 hours. The changes here observed are greater than those found in normal animals (Jørgensen), which naturally is due to the fact that the phosphorus excretion is inhibited. This rise is then followed by a fall, and this is not found to be lower in the experimental animals than in the controls.

Also serum calcium shows a tendency to increase but not in so pronounced a degree. Two of the animals, Nos. 80 and 82, showed no rise whatever, while Nos. 84 and 105 showed a distinct increase, about 16 %. The height of the increase in serum calcium appears to come a little later than that of serum phosphorus. The increase in the experimental animals is greater than the increase observed in similar experiments on normal animals (Jørgensen). This increase is followed by a moderate fall, which in no instance reaches values lower than those observed in the controls.

These experiments thus show that the parathyroid hormone is able directly to produce hyperphosphatemia and hypercalcemia — which is not keeping with the view of the parathyroid effect advocated by Albright and collaborators.

Summary.

1. In nephrectomized rabbits the writer has demonstrated a considerable rise in serum phosphorus and a smaller increase in serum calcium after parathyroid injections of 25 and 50 units.

2. These findings are taken to indicate that the parathyroid hormone is able directly to induce hyperphosphatemia and hypercalcemia.

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DERMATOMYOSITIS-LIKE CASE OF PLASMOCYTOMA WITH ENORMOUS HYALINE DEPOSITS (PARAMYLOID)

By *Konrad S. Jorgensen.*

Received for publication Sept. 2, 1944).

The clinical diagnosis of plasmacytoma may often be difficult, especially when massive hyaline and amyloid deposits dominate the picture of the lesion, suggesting an erroneous diagnosis. Nor does autopsy always give satisfactory information about the true nature of the lesion, as the plasmacytoma may be exceedingly difficult to locate. This leaves the characteristic amorphous deposits — partly hyaline and partly amyloid of character — in areas where true amyloid never is seen. A decade ago there was some tendency to interpret cases of this kind as an entirely new disease — *e. g.*, Lubarsch, who in one of his cases even mentions the abundant amount of plasma cells in the bone-marrow, without realizing their pathogenetic significance. Not until the last years was it fully realized (Magnus Levy, Apitz) that such deposits apparently are encountered only in connection with plasmacytoma and that they constitute one of the peculiar changes in the protein metabolism associated with this disease.

As so far no instance of this kind has been reported from this country, it will be appropriate to present the following case, the true character of which was very difficult to ascertain.

Case Record.

The patient was a woman, 43 years old. She was a seamstress, living under rather poor conditions, exposed to cold and draught, hard work and poor nutrition.

Past History: Menstruation commenced at the age of 17, always regular, 4/23 days. No parturition or abortion. At the age of 20, diphtheria, without complications. At the age of 37, prepatellar bursitis.

Present Illnes. — In 1941 the patient commenced feeling tired



Fig. 1.

Photo of the patient on 20/1/42.

Note the emaciation, muscular atrophy and hygromas over both shoulders and left sternoclavicular joint.

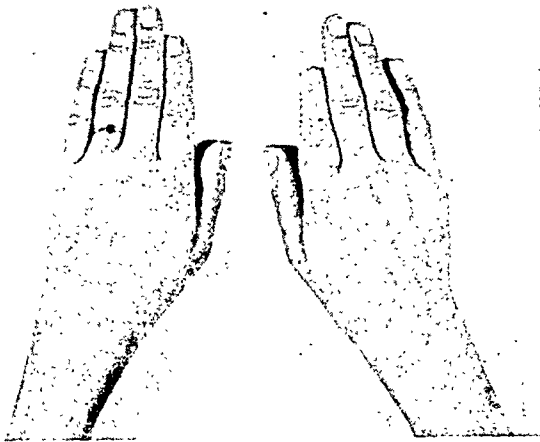


Fig. 2.

Hands of the patient, on 20/1/42. Note swelling of the wrists and the atrophic pigmented skin.

and losing weight. During that summer, small indolent nodules (size of hemp seed) began to appear round the eyes. At the same time, the patient had pain in the right hip, knee and ankle, mostly at night. There was some swelling and rigidity of these joints in the morning.

Gradually most of the joints, on both sides, became painful; she

lost her appetite completely and became increasingly emaciated. In November-December soft indolent intumescences developed over both shoulders. As gradually she was greatly distressed by the joint pains, on 17/12/41 she applied to the *Medical Out-patient Clinic* of the Kom-mune Hospital, which referred to her to the *Surgical Out-patient Clinic* — on account of the intumescences over both shoulders. Here puncture (in two seances) was performed of the hygromas (size of hen's egg) of both subdeltoid bursae, with evacuation of thin yellowish fluid, which was not examined further.

On 16/1/42 the patient was admitted to the *Physiurgic Dep.* of the hospital for treatment of her rheumatic affection.

Physical Exam. — The patient was found to be asthenic and emaciated. The skin was atrophic and tight, with scattered small areas of pigmentation and telangiectasis, especially on the face and upper arms.

Face: Numerous close-packed, papillomatous excrescences (size of hemp-seed) on the eyelids and round the eyes, mostly yellowish-brown in color, firm, of fibromatous consistency. A few were reddish-blue angiomas.

Eyes: Pupils normal. *Ears and Nose:* No abnormality. *Mouth:* Mucous membrane normal; tongue rather smooth, mobile; full prothesis. *Throat:* No abnormality.

Neck: No enlargement of lymph glands. Thyroid not enlarged.

Trunk: Moderate atrophy of the musculature, particularly pronounced in the shoulder girdle. Indolent hygromas (size of hen's egg) over both shoulder-joints. Plum-sized hygroma over the left sternoclavicular joint.

Auscultation of the Heart and Lungs: Normal findings. *Abdomen:* No abnormality. *Spinal column:* Normal.

Upper Extremities: Pain on movements in both shoulder-joints, swelling and tenderness of wrists, also of a couple of finger-joints of both hands.

Lower Extremities: Pain on movement, especially in the right hip. Crepitation in the knees; swelling and tenderness of the right ankle and a couple of digital joints.

Electrocardiography: No abnormality.

Laboratory Examination, see Table 1. In particular, no albumin in the urine, and a sedimentation rate of 4 mm.

During her stay in this department the patient complained of insomnia, partly from pain in the joint (not pain in the bones), partly from paresthesias, localized to the extremities as acroparesthesias.

For cosmetic reasons, cauterization of the small tumors round the eyes was performed (by the Skin Clinic) but they recurred very rapidly. One of these tumors was extirpated for microscopy (interpreted as colloid milium). On 23/4/42 a soft, fluctuating, tumor of hygroma character (size of mandarin) was found over the sacrum.

After 4 months of treatment with salazopyrin and sanocrysin, no sign of improvement. As further the standard metabolism was found to be slightly increased (+39, +34), the case was taken to be of hyperthyrotic character, on which account the patient was transferred to the *Surgical Department V*.

Clinically it was not possible to demonstrate any goiter. Circumference of neck/wrist = 13/16, but as the metabolism kept being increased, the patient was given preliminary diiodotyrosin treatment and on 11/5/43 subtotal thyroidectomy was performed. At the operation the thyroid was found practically to be of normal size. Microscopy of the resected glandular tissue showed no changes typical of hyperthyroidism, merely a rather pronounced round-cell infiltration.

Control examination two weeks after the operation showed a metabolic rate of +20 and a normal serum calcium concentration of 9.8 mg. %.

The patient was discharged for recreation in a convalescent home, during which she felt very poorly. The joint pains increased in intensity and the patient was strikingly inconvenienced by stiffness of the joints. Further, now pain and tenderness developed also in the musculature, but there was no tenderness of the bones. She complained a good deal of loss of sensation in the fingers and toes.

When, six weeks after her discharge, the patient tried to lift a heavy suitcase, she suddenly had an intense pain in the right hip, on which she was no longer able to support herself.

She was then readmitted to the *Physiurgic Dep.* of the hospital with all signs of fracture of the neck of the right femur: shortening of 2 cm., distinct tenderness of the trochanteric region, and maximal outward rotation.

Roentgenography revealed an almost medial fracture of the neck on the basis of a destructive process on the inferior aspect of the neck. The osseous tissue of the entire neck of the femur was cotton-like, with effaced structures; the head was sclerotic.

On 20/7/42 the patient was transferred to the *Surgical Dep. V* where extension was applied to the hip.

Physical examination: Skin atrophic, scaling, especially on the extensor aspect of the extremities. On the face, chest and upper extremities the polymorphous »map-like« picture of the skin was dominated by erythematous areas, brownish pigmentations and small telangiectases. The skin was firmly attached to the underlying structures, thin and glossy. No changes in the crop of small firm tumors (millet- to hemp-seed size) round the eyes and over the left clavicle.

Musculature: Very atrophic throughout and tender to touch. The patient complained greatly of pain in the muscles and joints when she tried to move the extremities and neck. Here and there — especially in the musculature of the neck, in the recti abdominis, and

in the lacuna musculorum femoris — indolent firm intumescences (bean to nut-kernel size).

Voice hoarse.

Mouth: The inside of the lips presented several yellowish-white firm intumescences (size of millet-seed) partly solitary, partly confluent, making the mucous membrane appear nodular. The tongue was smooth, deeply furrowed, of firm consistency, movable but slightly.

Trunk: No change in the aforementioned soft intumescences in the deltoid regions and over the sternoclavicular joint.

Auscultation of the Heart and Lungs: Normal findings. *Abdomen:* No abnormality.

The extremities showed reduced mobility in all joints. Neurological examination, as far as practicable for the joint and muscle pain, showed no abnormality.

Urine: Slight albuminuria; no Bence-Johnes protein.

Sedimentation rate now increased to 28 mm.

Because of the spontaneous fracture the calcium concentration of the blood was examined and showed a moderate constant increase to 13.4—14.4 mg. %, whereas there was no rise in plasma phosphorus.

Roentgenography of the bones was performed at the same time. The *spinal column* and the *skull* showed normal conditions as to calcium content and structure.

Long bones of the extremities, ribs and sternum showed nowhere any destructive processes of similar character as had been demonstrated in the right hip. On the other hand, the humerus as well as the femur presented a somewhat peculiar net-like structure in the proximal end with tiny areas (size of millet-seed) of rarefaction without marginal reaction. These changes did not resemble any found in other lesions, especially plasmocytoma.

This diagnosis seemed rather probable, however, as sternal puncture on 1/8/42 showed 20 % plasma cells (mononuclear). Formalin gel reaction gave normal findings, however, and the albuminuria was transitory, showing at no time the typical Bence-Johnes protein.

For further establishment of the diagnosis, test drilling was performed on one of the above-mentioned spots of rarefaction in the humerus. This showed no sign of plasma cells, whereas the bone appeared as markedly hyalinized connective tissue with extensive calcification; there was no evidence of genuine osseous tissue although the drill really entered the bone.

Finally, on 1/8/42, one of the small tumors in the lacuna musculorum was excised. The muscles were light in color, firm, somewhat atrophic. The extirpated »tumor« proved to be a lymph gland with slight inflammatory changes.

Towards the last the patient was lying apathetic, distressed with pain when she tried to move, and with increasing hoarseness and



Fig. 3.

X-ray photo of the right hip on 17/7/42.



Fig. 4.

X-ray photo of the right hip on 29/9/42.

dysphagia. The destructive process in the right hip was progressing, so that on 24/9/42 the entire neck of the femur was found to undergo dissolution, being only half as wide as before with fluffy margins; a couple of small »woolly« pieces of bone were attached to the

sclerotic head, which otherwise had lost all connection with the rest of the bone.

The hygromas subsided, but the infiltrations of the tongue and buccal mucosa increased considerably with the formation of whitish protruding plaques (up to the size of half a walnut) on the lips and at the corners of the mouth. The tongue became quite firm, with nodular infiltrations at the margin of the anterior two-thirds, while its root appeared to be firmly attached to the hyoid bone by tumor masses, which together with the mandibular lymph glands and the larynx formed a large firm conglomerate. There was no definite abnormality in the larynx itself.

The patient, who at no time had been febrile, became extremely debilitated and ceased moving almost completely. At last, there was a rise in temperature due to a pneumonic process that terminated fatally on 9/10/42.

Epicrisis: A woman, 43 years old, without any preceding complaint becomes ill suddenly and in the course of the following year she presents a most polymorphous clinical picture, with loss of weight, pains in the joint and skin changes consisting in atrophy, pigmentations, telangiectases and small, firm, skin tumors. Large hygromas are formed over a couple of the joints; subsequently muscular atrophy and tenderness developed. On account of an increased metabolic rate, subtotal thyroidectomy is performed, without resulting in any particular decrease in the metabolism. Under increasing joint and muscle pains, spontaneous fracture of the neck of the right femur takes place on account of destruction of the bone. Increase in serum Ca and 20 % plasma cells in the sternal punctate are suggestive of the diagnosis plasmocytoma, whereas there is no evidence of concomitant paraproteinemia or paraproteinuria. Under increasing general debility and apathy, small intumescences appear in the muscles, besides pronounced infiltration of the tongue and oral mucosa. Finally, the patient is immobilized completely by her affection and dies with pneumonia.

Differential Diagnosis.

If now, on the basis of the clinical data, we attempt a differential diagnostic analysis of this case, it will be noticed that, in the course of events, several lesions had to be considered, namely: primary progressive polyarthrititis, hyperthyroidism, hyperparathyroidism, till finally we arrived at the rather certain diagnosis of plasmocytoma, even though this case in several respects reminds strongly of dermatomyositis.*) The differentiation between the two last-mentioned lesions will be reviewed in the symptomatology.

*) As to the typical clinical picture of dermatomyositis, see Herman Nielsen's »Forelæsninger over klinisk Endokrinologi III« or the classical description given by Steiner (1905).

Symptomatology.

Joint complaints of polyarthritic character have been reported in cases of plasmocytoma but have not received much attention. Magnus Levy (1938) was able to find only a few cases in the literature, and in 7 of these the joint complaints were associated with amyloid tumors — he had seen one of these cases himself. In dermatomyositis, on the other hand, joint complaints are rather well known, especially in the chronic form of the lesion (*e. g.* Fahr; Kinney & Maher). Fahr has even set up a special group in which he claims that polyarthritic changes are of pathogenetic significance to the development of dermatomyositis.

Muscular changes with atrophy, pain, stiffness and tenderness, localized especially to the musculature of the upper extremities and the trunk are symptoms suggestive of dermatomyositis, in which the muscular phenomena are of decisive significance as compared to the skin changes — as has been emphasized in particular by Fahr and by Marcus & Weinstein. Heyn has described patients with dermatomyositis as lying distressed and apathetic as in plaster casts when the lesion is at the end of its chronic stage. This is a picture that would apply very well to our patient, who in the last months of her life was altogether incapable of doing anything herself and complained at the least attempt at passive motions. In plasmocytoma this is a most infrequent phenomenon, for here the pains in the bones constitute the predominant feature. Still, as will be pointed out below, it is rather conceivable that a similar clinical picture may result from enormous deposits of amyloid.

The *skin changes* present some features of characteristic nature, namely: the firmly attached and stiff skin of the extremities, and the small firm skin tumors on the face and neck. In dermatomyositis we may meet with skin changes of practically every kind; atrophy and pigmentations are typical, and so are telangiectases, which indeed were present in this case too. On the other hand, the presence of oedema is characteristic of dermatomyositis at a certain stage — and no oedema was observed in our patients. Skin tumors of similar character as those observed here have been reported in dermatomyositis by Urbach and Kinney & Maher and in a couple of other cases too, but they are no characteristic finding. In plasmocytoma the skin changes are not typical as a rule. Persistent eczema-like lesions have been observed. It is only in rare cases where so-called amyloid is deposited in the skin that we meet with rather polymorphous changes as pigmentations or nodules (Freudenthal, Gottron) sometimes of quite the same character as seen in our case (Speares; Piney; Lubarsch).

Changes in the mucous membranes and tongue of the character

seen in the present case are not common phenomena in plasmocytoma. In the so-called amyloid deposits, however, quite similar features may be encountered (Lubarsch; Zeehuysen; Glaus; Paul; Ritter). Dermatomyositis may be associated with rather pronounced changes in the mucous membranes (such cases have even been set up as a special group: dermato-muco-myositis) but never of such a character as seen in our case. These changes are accompanied by the *hoarseness*, due to changes in the mucous membrane of the larynx. In dermatomyositis marked changes in this mucous membrane have been reported by Karlmark.

Bone Changes. — Osseous changes in dermatomyositis have been described only in one instance (Drouet, Florentin, Verain & Girard), in which spontaneous fractures together with extensive osteoporosis were demonstrated — but no distinct destruction of bone in any place — besides pronounced articular changes, with ankylosis of the elbow. In this case probably the diagnosis was somewhat dubious (see below).

In our case the roentgenographically demonstrated bone destruction is highly suggestive of plasmocytoma, as there might have been a solitary process in the right hip-joint. The aforementioned spots of rarefaction, which were found especially in the humerus, proved on drilling (and, later, on autopsy) not to contain plasma cells. The slightly peculiar bone structure corresponds rather to moderate osteoporosis, and this configuration of the bone structure may be found also in certain endocrine lesions (*e. g.*, hyperthyroidism).

The appearance of *hygromas* is not typical of either lesion, at present it will be put down as an accidental finding.

General Symptoms. — Loss of weight (bordering on cachexia), loss of appetite and anemia are not particularly characteristic, being present in a number of lesions. Still it is worth noting that the anemia was exceedingly persistent, and that it recurred at once on discontinuance of the iron therapy. This, I think, may be taken to support the diagnosis plasmocytoma.

Clinical Laboratory Findings.

Hypermetabolism is generally present in plasmocytoma, usually in a moderate degree, with an increase of 10—15 % (Magnus-Levy). In dermatomyositis, on the other hand, a rather considerable rise in the metabolic rate has been found repeatedly — *e. g.*, Marcus & Weinstein (+ 31 %); Uhrbach (+ 41 %); Haxthausen (+ 39 %).

Hypercalcemia may likewise be found in either lesion. In plasmocytoma it is a rather obligatory finding (up to 20 %), and in derma-

Table 1.
Clinical Laboratory Findings.

	1941 17/12	1942 19/1	15/2	9/4	5/5	25/5	16/7	19/7	22/7	30/7	5/8	22/8
Height, cm.....	173											
Weight, kg.....	53.4	50.6	51.0			53.2	49.8					38.0
Pulse.....	70	70	90	82	96	94	100					
Blood pressure.....		160/85										
Metabolism %.....		139	134	128	128	120						
Urine.....	Normal		— Alb.		— Alb.		+ Alb.	+ Alb.	— Alb.		— Alb.	— Alb.
Feces.....			— Bz.								+ Bz.	+ + Bz.
Sedimentation rate, mm.	4						28					
Formalin gel.....							—	—		—	—	
Hemoglobin %.....		82	55	73			63		87		66	81
Color index.....			1.1				0.9					
Serum Ca mg. %.....					10.0	9.8		13.4	14.4	13.6		
Urine Ca.....									46.6	52.2		
Plasma P.....									4.6	3.6		
Urine P.....									99	108		
Diuresis.....									1400	900		
Antistreptolysin.....	80	80										
Phosphatase/100 ml.							78					

In addition: Wassermann negative, gonoreaction negative, serum uric acid normal. Differential count normal.

tomyositis it is no unknown phenomenon, having been reported by Drouet, by Ingram and by Draganesco.

The *sedimentation rate* in our case rose slowly from 4 to 47 mm., but at no time did it reach the formidable heights characteristic of plasmocytoma; it kept at moderate levels as is generally seen in dermatomyositis.

Likewise it is worth pointing out that the formalin gel reaction turned out negative, on which account no additional serum protein determinations were carried out. In this connection it is to be mentioned too that at no time was it possible to demonstrate the presence of Bence-Johnes protein in the urine.

The *blood picture* showed no particular abnormality, the two differential counts performed giving normal values.

Urine: Twice, at an interval of 3 days, a small amount of albumin was present, but it presented nothing characteristic, and it did not recur throughout the course of illness. The Ca and P values were practically normal.

Conclusion from the Clinical Findings.

The clinical picture of this case presents many features common

to dermatomyositis. Still the bone changes and the findings in the sternal punctate suggest that here we are dealing with a form of plasmocytoma even though at no point of time was there demonstrated any paraproteinemia or paraproteinuria (Apitz), and the sedimentation rate kept at very moderate levels.

Pathologic-anatomical Findings.

Biopsies: Two of the above-mentioned *hemp-seed-sized, firm, sessile tumors* were removed from the skin; they showed the same features. Beneath a thin layer of squamous epithelium (5—6 cells in height), nodular masses were seen to consist of a trabecular, amorphous, substance of hyaline character (no test for amyloid performed); these nodules extended from the papillary layer down in the subcutis. There was no sign of any inflammatory process.

Drilling biopsy from the humerus showed no tissue of osseous character, although the drill had entered the bone. The specimen consisted of markedly hyalinized connective tissue with very few cells and considerable calcium deposits. No plasma cells.

Lymph gland from the femur showed desquamative lymphadenitis with large germinating centers, considerable increase in and desquamation of the sinus epithelium. In many places the interstitial connective tissue was somewhat increased, hyalinized and the site of dense dark streaks of cells as in chronic irritation. No plasma cells. Blood vessels normal.

Pieces of the resected *thyroid gland* showed nowhere changes as



Fig. 5.

Biopsy of sessile skin tumor Hematoxylin (Hansen)-eosin.

seen in exophthalmic goiter, but rather large round-cell infiltrations in the interstitial tissue.

Autopsy, on 13/10/42 (Pathological Institute):

As the macroscopic autopsy findings on several points corresponded to the clinical description, they will here be cited but briefly.

The body was that of an emaciated cachectic patient with yellowish-brown pigmentations, reminding in outline of a map, particularly pronounced on the extensor aspects of the extremities.

Tongue firm, thick, with hypertrophic papillae.

Pleura: Thin fibrinous patches.

Lungs: Voluminous, slightly nodular, congested, with oedema and typical pneumonic foci. Pus in the bronchi.

Heart: Small, firm, with marked hypertrophy of the left ventricle. Aortic valves a little too rigid, with a couple of small nodules and fusion of the margins.

Stomach: Pyloric part firmly thickened, with thick and folded musculature and voluminous folds of the mucous membrane. The polyric ring hangs down into the duodenum like a red inflamed lip. These changes are sharply limited above and below.

Intestinal canal apparently normal.

Parenchymatous organs apparently normal.

Genitals normal.

Brain: No macroscopic abnormalities.

Blood vessels apparently normal.

Muscles: Flaccid and light in color. The rectus abdominis contains several firm, solid, nodes (from hazel-nut to pigeon egg in size), consisting of wavy, partly lamellar masses of hyaline character, proliferating to the sides, where it turns into lighter but otherwise apparently normal musculature.

Bones: Corresponding to the destructive process in the neck of the right femur, oedematous (myxomatous) fatty tissue with streaks and trabeculae of hyaline character. In the left shoulder-joint, beginning destruction of similar character. Otherwise no sign of bone destruction.

Microscopy: Section from 12 different specimens of tissue were examined.

1. *Hypophysis*: Normal findings.

2. *Adrenals*: No abnormalities except in one artery, which presented changes of the same character as described below.

3. *Kidney*: No changes typical of paraproteinuria; normal findings except for slight changes in the blood vessel.

4. *Pylorus, at transition to the fundus*: Marked vascular changes. In the muscularis mucosae, areas — »islands« — or plaques of hyaline-like degenerative swelling of the musculature and, presumably, connective tissue. This hyaline-like tissue is trabecular, arranged in flakes or lumps, in unstained sections appearing shiny homogeneous



Fig. 6.

Part of the pyloric musculature. v. Gieson-Hansen.

in stained sections more or less markedly eosinophil, taking a rose color when stained with acid fuchsin.

5. *Bone-marrow*: Markedly hyperplastic with abundant erythropoiesis and granulopoiesis. No definite plasma cells.

6. *Lymph glands*: No abnormality except for a moderate increase in interstitial hyaline connective tissue.

7. *Tissue from the site of the fracture in the right hip*.

8. *Tissue from the left shoulder*: No tissue elements reminding of osseous tissue. A few small nodules of hyaline in the periosteum; otherwise collagen connective tissue and adipose tissue interspersed with hyaline trabeculae and streaks. In some areas, an amorphous substance with a few connective tissue cells, apparently necrotic, with scattered calcifications.

9. *Node from the rectus abdominis*; and



Fig. 7.

Musculature from a node in the rectus abdominis.



Fig. 8.

Small artery in the wall of the stomach.

10. *Tissue from the neck* (thyroid parathyroid region): In the node streaks of degenerated muscle fibers surrounding nodular, solid, wavy, partly lamellar, hyaline masses interspersed with clusters of lymphocytes and multinuclear giant-cells (of same type as foreign-body giant-cells) in streaks of collagen connective tissue. In the cervical musculature hyaline nodules of the same character as above, with giant-cells, surrounded by thin degenerated muscle fibers, here and there in musculature of normal appearance.

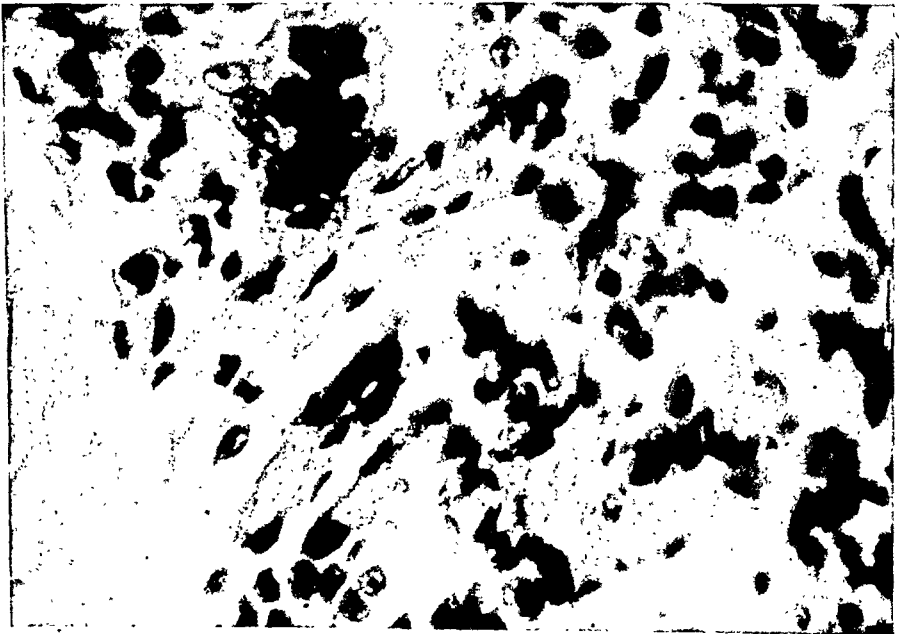


Fig. 9.

Same specimen as Fig. 7. High magnif. Foreign-body giant-cells in the musculature.

11. *Tissue from thoracic vertebra:* All bone trabeculae are quite narrow, with few cells, rather suggestive of osteoporosis. Here too the bone marrow appears normal.

12. *Joint capsule from the wrist:* Nodules of hyaline-like degeneration, surrounded by normal connective tissue with moderate lymphocyte infiltration.

Blood vessels: Many of the small arteries show considerable changes: endothelial proliferation of the same character as seen in obliterating thromboangitis; also, here and there, smaller and larger hyaline-like nodules in the media; in some places the entire media is transformed into an amorphous substance with few connective tissue cells, and here the media is enormously thickened. Such changes are seen in most of the organs.

In order to establish the character of the hyaline-like substance, tests for *amyloid reaction* were performed, as this substance in some places was so conspicuously proliferating, »tumor-like«, as to be suspicious of amyloid. In every instance, however, the iodine reaction and Jürgens' methyl violet reaction turned out negative. On the other hand, there was a distinct positive amyloid reaction with Congo red (after Bennhold) though only in the above-mentioned pronounced vascular changes, and nowhere else. The skin was not examined for amyloid.

Discussion.

Correlating the clinical features of the lesion in this case with the autopsy findings, we now see that really there would be but a meager foundation for the diagnosis dermatomyositis, and that this diagnosis primarily would be due to a certain external relation between the two affections. As dermatomyositis merely is a syndrome, the etiological factors of which have not yet been established (»an acute, subacute or chronic disease of unknown origin«, Steiner; Langmead; Weinstein & Marcus; and others) it is not entirely excluded that several cases described under this diagnosis may have been of similar character as the case here presented, in particular as autopsy has been reported only in very few instances of dermatomyositis.

In the present case, however, the striking changes of hyaline nature show quite plainly that here we are dealing with another lesion. Hyaline degeneration of muscles, skin and organs is found in dermatomyositis, it is true, but never in such a marked degree as in the present case. On the other hand, in the literature we meet with several cases that have a striking resemblance to ours (Wild 1886; Steinhaus 1902; and, especially Ritter 1908). A total of about 20 cases has been reported, in which the changes were just as extensive as

in our case. Thus, in 1929, Lubarsch gave a thorough description of 3 cases, but he too failed to realize the true nature of the affection as he mentioned them as instances of »ungewöhnlicher Amyloidablagerungen«, although he was aware that the substance here deposited gave an atypical amyloid reaction. In his comprehensive work on myelomatosis, Magnus Levy (1938) called attention to the fact that these deposits consisted of paramyloid — a substance excreted in myelomatosis (plasmocytoma). He also demonstrated that at any rate in one of the cases reported by Lubarsch there was a marked increase in the plasma cell content of the bone marrow.

Magnus Levy's paramyloid is characterized by being deposited in places where true amyloid is never seen, while it is not found at the typical sites for amyloidosis — in the parenchymatous organs (liver, spleen and kidney). This substance is amorphous, trabecular in structure, almost tumor-like in its accumulation (in some cases it is found as a solitary amyloid tumor, often of formidable dimensions). On section, in unstained state, it is shiny, glossy; on staining it is eosinophil; and it gives very uncertain, atypical, amyloid reactions, most often Congo red reaction, especially localized to the blood vessels — as observed already by Wild. Thus it is not practicable with certainty to decide whether one is dealing with amyloid or with hyaline. More likely it is a third substance, closely related to the other two, and perhaps forming a transitional stage between them, as these substances are not well defined — and hyaline, for instance, is »neither a morphological nor a chemical, nor a development unit« (Lubarsch).

Recently, the nature of paramyloid has been investigated more thoroughly by Apitz (1940) who emphasizes that growing plasma cells constitute an indispensable prerequisite for the formation of paramyloid and that this substance as well as Bence-Johnes' protein may occur in intact bone-marrow — namely, in cases of extramedullary plasmocytoma. Paramyloid, Apitz claims, is formed parallel with Bence-Johnes' protein, it occurs together with hyperproteinemia, and it may possibly be looked upon as deposit from this condition. This does not apply to our case, however, in which no hyperproteinemia was observed, nor any particular degree of proteinuria. Furthermore, the relatively low sedimentation rate, which was followed throughout the course of the lesion, and the absence of changes in the kidneys have to be interpreted to this effect too. It seems rather likely that paramyloid may make its appearance as a solitary »tumor« but the present case gives no information as to whether it may appear without the presence of plasma cells.

Conclusion. In the present case the diagnosis »plasmacytoma« was made once — on the basis of the findings in the sternal punctate. On repeated biopsies as well as on autopsy, however, it was not practicable to demonstrate any abnormal presence of plasma cells in

the destructive bone processes or elsewhere in the organism. Still, the enormous deposit of a substance corresponding to the description of paramyloid suggests the persistent presence of this affection although it has not been possible to find the focus so that the lesion may have been plasmocytoma of extramedullary nature.

Summary.

A report is given of the peculiar case of a woman, 43 years old. Her condition reminded greatly of dermatomyositis with articular changes. In addition, however, she presented large hygromas and suffered a spontaneous fracture of the femur. Sternal puncture showed 20 % plasma cells, on which account the lesion was taken to be plasmocytoma. Autopsy revealed large hyaline-like masses in the mouth, stomach, muscles, skin and blood vessels, but no accumulation of plasma cells.

The hyaline-like substance was found in some places to give an atypical amyloid reaction, and morphologically it resembled amyloid, but it was not found in the typical sites of amyloidosis.

It is pointed out that previously about 20 cases of a similar character have been reported. It probably involves the so-called paramyloid deposits which are said to be typical of plasmocytoma, constituting a link in the characteristic changes in the metabolism encountered in this affection. It is emphasized that hyperproteinemia or Bence-Jones' protein in the urine could not be demonstrated at any time in the course of the present case. Finally, the failure to demonstrate the presence of plasma cells on autopsy is of interest.

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UNTERSUCHUNGEN ÜBER DIE KEIMTÖTENDE WIRKUNG EIN- UND MEHRBASIGER ALKOHOLE

Von O. Ornstein und K. G. Thorsson.

(Eingegangen bei der Redaktion am 27. September 1944.)

In einer Arbeit, welche sich mit der Theorie und Technik der Desinfektion geschlossener Räume befasst, haben Twort und Mitarbeiter Versuche mitgeteilt, durch in Luft zerteilte, keimtötende Mittel (Aerosole) die in einer geschlossenen Atmosphaere in Tröpfchen oder Staubteilchen schwebenden pathogenen Keime abzutöten (1). Unter den zahlreichen, geprüften Gemischen erwiesen sich in Propylenglykol gelöste Phenole besonders wirksam, was nach Ansicht der Autoren zum grossen Teile wenigstens auf der sehr langsamen Verdunstung der feineren Tröpfchen eines solchen Gemisches bei der Versprühung beruhen muss. Nebel dieser Art hielten sich bei Beobachtung durch das Ultramikroskop besonders lange in der Luft. Mit der Konzentration des Lösungsmittels beobachteten die Autoren ein Ansteigen der Teilchengrössen. Da Dämpfe der hier in Frage kommenden Stoffe — wohl ungesättigt — nach Ansicht der Autoren unwirksam sind, ist die Bestimmung der optimalen Teilchengrösse solcher Nebel sehr erwünscht. Rücksicht ist dabei zu nehmen auf die Destillationswirkung getrockneter Luft auf Phenole sowie auf das hygroskopische Verhalten der Glykole.

Platten, welche 5—15 Minuten nach Einwirkung des Aerosols auf die versprühten Keime für zwei Minuten in der Kammer exponiert wurden, erwiesen sich als keimfrei noch bei Konzentrationen des Entkeimungsmittels entsprechend 1 gr in 50 cbm Luft (berechnet auf 5—10-prozentige Lösungen von Hexylresorcin in Propylenglykol). Zur Feststellung der Wirkung erwies sich die Plattenexposition allen anderen Verfahren der Keimbestimmung bei weitem überlegen. Die Versuchstechnik bedarf aber nach Ansicht der Autoren genauerer Kontrolle und einer Standardisierung hinsichtlich der Zahl und Art der verwendeten Keime wie auch der wirksamen Dosen in Frage kom-

mender Mittel für jede Keimart und hinsichtlich der physikalischen Eigenschaften geeigneter Nebel.

Die in Frage kommenden Mittel dürfen in ihren wirksamen Grenzwerten weder reizend für die Haut oder Schleimhäute noch giftig sein. Sie müssen unsichtbar und geruchlos, sowie unschädlich für Werkstoffe wie Holz, Metall, Gewebe oder Farben sein. Sie dürfen schliesslich nicht flammbar sein, müssen eine gewisse Beständigkeit in der Atmosphaere besitzen sowie Keime rapide abtöten. *Wasserlöslichkeit des Entkeimungsmittels und des Vehikels (Lösungsmittels) sind bei Luftsterilisation wahrscheinlich erwünscht* ganz wie auf anderen Gebieten der Desinfektion u. zw. *Wasserlöslichkeit bei geringer Neigung zur Verflüchtigung*. Hohe Rideal-Walkerwerte erwiesen sich oft als trügerisch. Am günstigsten schienen Teilchengrössen von $0.5-1.0 \mu$ zu wirken, nach Messungen der Autoren nur etwa 5 vom Hunderl der versprühten Aerosole. Sind reichlich organische Substanzen z. B. Speichel zugegen, so steigen die zur Sterilisierung der Atmosphaere benötigten Konzentrationen der Mittel auf das Vielfache (2).

In einer späteren Arbeit (3) wird der Einfluss der Luftfeuchtigkeit auf die keimtötende Wirkung wasserunlöslicher, durch Versprühung oder Hitzeverflüchtigung in Luft verteilter Stoffe, wie Weihrauch, Myrrhen, Styrax, Benzoin u. a., betont. Ferner war mechanische Versprühung von Hexylresorcin in Propylenglykol wirksamer als Hitzeverflüchtigung (Verdampfung), Resorcin in Propylenglykol dagegen wirksamer bei Hitzeverflüchtigung. Bei steigender relativer Luftfeuchtigkeit nimmt die keimtötende Wirkung vernebelter, wasserunlöslicher Phenole zu, ein zu grosser Sättigungsgrad führt aber wieder zu deren Abnahme.

Die Schlussätze der Autoren lauten: »No precise recommendation can be given as to percentage relative humidity, germicide and method of generating aerosol in order to obtain maximum disinfection of air, until more data are available concerning the chemical composition and physical behaviour of the operative germicide particles.«

Robertson, Bigg, Miller und Baker (4) fanden, dass Glykole an und für sich als Aerosole stark keimtötend wirken. Mit einem Gewichtsteil Propylenglykol in zwei Millionen Volumteilen Luft erzielten sie in ihren Kammerversuchen vollständige Sterilisation einer Atmosphaere, welche 200000 weisse Staphylokokken im Liter Luft enthielt u. zw. einige Sekunden nach Einführung des Aerosols. Gleiche Wirkung konnten sie gegen Pneumococcus I und III, Streptoc. haemolytic. und viridans, Bact. coli und Microc. catarrhalis feststellen. Die untersuchten Glykole, 1.3- und 1.2-Propylenglykol sowie Äthylenglykol, waren gleich wirksam, weniger Glycerin. Dass die Glykole auch wirklich abtötend wirken, konnten sie bestätigen. Der Mechanismus der Glykolwirkung sowie die Verträglichkeit der Glykole als Aerosole bei längerer Exposition von Versuchstieren sind zwar noch nicht abschliessend geklärt, ihre starke keimtötende Wirkung aber wegen der

überaus geringen Giftigkeit von grosser Bedeutung. Konnten doch *Hanslick, Newman, van Winkle, Lehman* und *Kennedy* (5) nachweisen, dass Propylenglykol intravenös verabfolgt kaum ein Viertel so giftig ist wie Aethylalkohol; bei mittleren über längere Zeiträume hin oral einverleibten Mengen entbehrt es überhaupt jeder nachweislichen Giftwirkung; vielmehr kommt ihm glykogenspeichernde Wirkung zu, zeigen die Versuchstiere daher schnelleres Wachstum und erreichen höheres Gewicht und grössere dynamische Leistungsfähigkeit als die Kontrollen. Die Autoren empfehlen deshalb für den inneren Gebrauch in der Kinderpraxis als geeignetes Vehikel für Nahrungsmittel und medizinische Produkte Propylenglykol an Stelle von Aethylalkohol.

Neuerdings haben *Stokes* und *Henle* (6) Propylenglykol in gleicher Konzentration wie die oben genannten Autoren (4) gegen mäusevirulentes Influenza-A-Virus als wirksam befunden. In Allantoisflüssigkeit von Hühnerembryonen gewachsenes Virus wurde in einem grösseren Krankenzimmer versprüht und durch hitzeverdampftes Propylenglykol seiner Wirksamkeit beraubt.

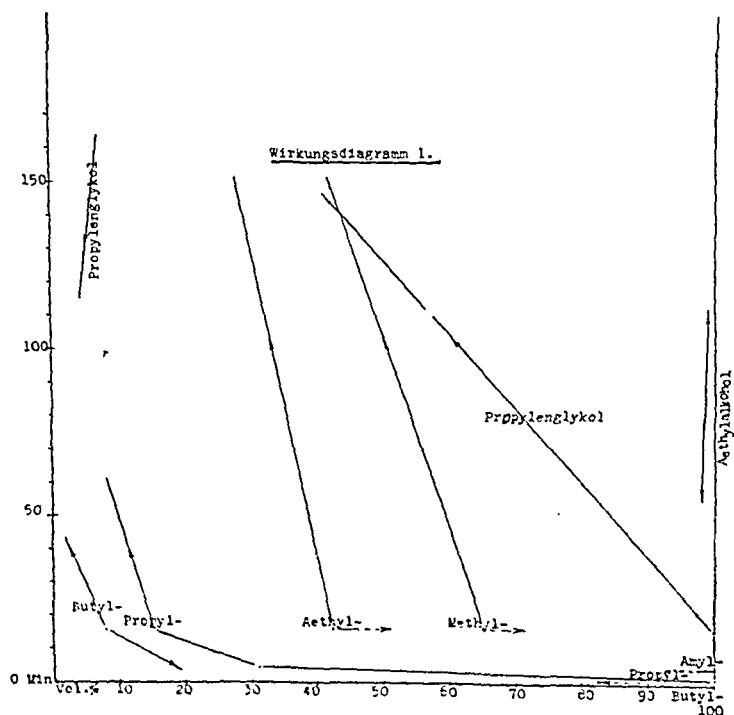
Auf die grundlegende ältere Litteratur über die Wirkung flüchtiger Dämpfe organischer Stoffe und deren Beziehung zur Temperatur und zum Sättigungsgrade der gasförmigen Aggregate sowie zum Wassergehalte der Ausgangslösungen sei hier nur kurz verwiesen. Den Dämpfen des Aethyl-, Propyl-, Butyl- und Amylalkohol eignet danach eine erhebliche sterilisierende Wirkung auf pathogene Keime und Sporen, welche durch Zusätze von Wasser bzw. Phenol noch erheblich verstärkt werden kann (7).

Auf die von Twort und seinen Mitarbeitern berührten Fragen der Wirkung gasförmiger oder flüssiger Aggregate, der Bedeutung des Wassergehalts der Atmosphaere für die Wirkung sowie einer endgültigen Grenzwertbestimmung der fraglichen Stoffe oder Stoffgemische sind wir in unseren Versuchen nicht näher eingegangen. Das kann nur in methodischen Versuchen in zureichendem Masse geschehen. Uns kam es vielmehr darauf an, die von diesen Autoren als wirksam befundenen Glykole einer näheren Prüfung zu unterziehen. Wir wollten insbesondere feststellen, wie die einer nahe verwandten chemischen Gruppe zugehörigen ein- und zweiwertigen Alkohole sich einerseits als reine, mehr oder weniger konzentrierte flüssige Stoffe in vitro, anderseits als »Aerosole« in ihrer keimtötenden Wirkung verhielten. Auf die Wirkung flüchtiger, aber nicht wasserlöslicher Körper sowie von Gemischen mit solchen sind wir hier nicht eingegangen. Leider stand uns nur 1.2-Propylenglykol zu Gebote.

Wir haben also zunächst eine Reihe niederer Alkohole in ihrer Wirkung auf »Keimträger« mit der des Propylenglykol verglichen. Die Überprüfung der für die niederen Alkohole durch die Untersuchungen *Wirgins, M. Christiansens* und *Gregersens* ((7) S. 1087 ff.) bekannten Verhältnisse im Vergleiche mit der Wirkung des Propylenglykol ergaben, dass dieser an die unterste Stelle der steigende Reihe Methyl-,

Aethyl-, Amyl-, Propyl- und Butylalkohol zu setzen war. Dabei muss aber hervorgehoben werden, dass die Wirkung des konzentrierten Propylenglykol der des Methyl- und Aethylalkohol doch überlegen sein dürfte und hier zwischen Aethyl- und Amylalkohol in die steigende Reihe eingeschaltet werden muss. Die verzögerte Wirkung der beiden ersteren Alkohole tritt hierbei in unseren Versuchen deshalb nicht so klar hervor, weil wir im Gegensatz insbesondere zu Christiansen nicht mit trockenen Keimträgern arbeiteten. Während Aethyl- und Metylalkohol etwa zwischen 40- bzw. 70-prozentiger und annähernd 100-prozentiger Konzentration ihrem Wirkungsoptimum zustreben, Propylalkohol dagegen schon von 30-, Butylalkohol schon von weniger als 10-prozentiger Lösung ab, läuft die Wirkungskurve des Propylenglykol von etwa 30-prozentiger Lösung ab fast geradlinig auf ihr Maximum in konzentrierter Lösung zu und übertrifft so — in mittleren Konzentrationen bedeutend schwächer als Methyl- und Aethylalkohol — beide in reiner Form an Wirkung (Wirkungsdiagramm 1).

In konzentrierter, analysenreiner Lösung wirkte Propylenglykol erst nach 16, Isoamylalkohol nach 4 Minuten, Propyl- und Butylalkohol schon nach 1 Minute abtötend auf Staphylokokken. Bei den hier zu Grunde liegenden Versuchen wurden dicke, fünf cm lange, durch Kochen sterilisierte Leinfäden als Keimträger verwendet. Die-



Wirkungsdiagramm 1.

selben wurde in einer Staphylokokkenemulsion entsprechend einer Öse Kultur auf 50 cm³ Seewasser während etwa zehn Minuten beladen, einen Augenblick auf sterilem Filtrierpapier abgetropft und sofort in die zu prüfenden Alkohole übergeführt. Nach bestimmten Zeiten u. zw. nach 1, 4, 16, 64, 256 Minuten, u. U. auch nach 24 Stunden wurden die Fäden nach kurzem Abtropfen in flüssigen, bis nahe an die Erstarrungsgrenze abgekühlten Agar übergeführt, und die Keime unter Umschwenken in der Gussplatte verteilt. Die Auszählung erfolgte nach 24 stündiger Bebrütung und achttägiger Beobachtung.

Tabelle 1.

Propyl-, Butyl-, Amylalkohol und Propylenglykol in konzentrierter analysenreiner Lösung. Wirkung gegen *Staphylococcus aureus* an feuchten Leinfäden als Keimträgern. Auszählung der Gussplatten nach 8 Tagen:

Einwirkungszeit:	1	4	16	64 Minuten
Aethylalkohol	.	.	11	> 2
Propylalkohol	0	0	0	0
Pr. Butylalkohol	0	0	0	0
Isoamylalkohol	180	0	0	0
1.2-Propylenglykol	2415	520	0	0
Wasserkontrolle	1865	2535	975	611
Einsaatkontrolle				16315

Diese Ergebnisse haben wir mit dem Verhalten der genannten Alkohole und des Propylenglykol unter den Bedingungen verglichen, wie sie aus den Versuchen von Twort und Robertson und deren Mitarbeitern hervorgehen: Eine Kammer von 3·3·5 dm entsprechend 45 l Inhalt und mit gut abgedichteter Tür wurde am Boden mit einem Schieber versehen. Unterhalb desselben befand sich ein Metallcylinder von 10 cm lichter Weite und 20 cm Länge, der mit einem hermetisch schliessenden Deckel mit Bajonnetverschluss zur Aufnahme von Nährbodenplatten versehen war. Die Sprühinfektionsversuche wurden so angestellt, dass Aufschwemmungen von Keimen mit einer einfachen Sprühvorrichtung, wie sie zur Asthmabehandlung Verwendung findet, von unten durch den Cylinder in die Kammer eingeblasen wurden. Dem besonders anfänglich raschen und keimreichen Falle der grösseren folgt ein langsames und spärlicheres Absinken der mittleren und kleineren Tröpfchen. Zur Registrierung der allmählichen Abnahme der Keime ist es zweckmässig, entsprechend dem Vorgange Tworts zu verfahren d. h. einmal während der ganzen Zeit des Versuchs u. zw. während höchstens einer Stunde am Boden der Kammer Platten zu exponieren, zweitens aber zu verschiedenen Zeiten Teilexpositionen vorzunehmen. Abweichend von Twort haben wir am Boden des beschriebenen Cylinders Platten während der ersten Viertelstunde dreimal hintereinander für je fünf Minuten, dann für eine Viertelstunde, zuletzt für eine halbe Stunde exponiert. Die zusammengerechneten Koloniezahlen der Teilexpositionen entsprachen bei Kon-

trollversuchen mit mittleren Keimzahlen (etwa entsprechend 10000 Keimen im Liter Luftraum) ungefähr den mittels Dauerexposition bestimmbaren Mengen. Bei höheren Keimzahlen gaben die zusammengezählten Kolonien der Teilexpositionen ein genaueres Bild als die Dauerexposition, welche durch die grössere Keimdichte dann geringere Zahlen aufwies.

Wie schon in der Einleitung erwähnt, galten unsere ersten Versuche einem Vergleiche verschiedener in ihrer Wirkung wohldefinierter Alkohole mit dem in dieser Hinsicht bisher weniger bekannten Propylenglykol. Die englischen Autoren erwähnen nur nebenbei, dass ausser den in erster Linie geprüften Phenolen auch dem (nur als Vehikel wasserunlöslicher Körper gedachten) Propylenglykol eine gewisse antiseptische Wirkung zukomme. Im übrigen wird in den einschlägigen Arbeiten, auf welche hier Bezug genommen wurde, nichts dergleichen erwähnt. Es war daher bemerkenswert, dass der reine Propylenglykol eine recht erhebliche keimtötende Wirkung aufwies, welche die des konzentrierten Äthylalkohol jedenfalls übertraf. Die Versuche, in der geschlossenen Kammer verstreute, in kleinsten Tröpfchen oder Stäubchen schwebende Keime durch versprühte Alkohole zu beeinflussen, wurden nach dem Vorgange der Engländer in der Weise vorgenommen, dass zuerst der betreffende Alkohol, und eine Minute später die Keimemulsion versprüht wurden. Die Keimemulsion war in Liebigbouillon verteilt. Die benutzten Sprühapparate waren nach Möglichkeit geeicht. Da wir Bouillonaufschwemmungen benutzten, haben wir von Anfang an mit höheren Konzentrationen der geprüften Alkohole gearbeitet als die oben genannten Autoren. Die Temperaturen während der Versuchszeit haben wir notiert. Die Luftfeuchtigkeit haben wir in unseren Versuchen nicht bestimmt, werden aber diese Frage weiterhin noch streifen.

Die Kontrollversuche bilden die Grundlage für die Bewertung der Testversuche, in welchen die verschiedenen Alkohole in ihrer Wirkung auf bekeimte Atmosphären untersucht wurden. Je stärker dabei die Wirkung eines Mittels ist, um so grösser auch die Abnahme der Koloniezahlen, besonders bei der Dauerexposition. Für Kontroll- und Testversuche ergeben sich dabei, entsprechend deren Teilexpositionen, zwei Kurven der zu gleichen Zeiten noch vorhandenen Keimzahlen, ausgedrückt in Hundertteilen der gezählten Kolonien des Kontrollversuchs. Auf diese Weise wird die relative Keimverminderung in der Versuchszeit gut veranschaulicht. Die reziproken Werte einander entsprechender Prozentzahlen geben den jeweiligen Entkeimungsfaktor an. Wegen etwaiger Nachwirkung der in der Kammer versprühten Alkohole, und um die Kontrollversuche mit Bakterien allein nicht allzu günstig erscheinen zu lassen, haben wir diese meistens an den Schluss der Versuchsreihen verlegt und trotzdem feststellen können, dass innerhalb der vier bis fünf Stunden, welche eine Versuchsserie in Anspruch nahm, die Gesamtzahl der Keime in der

Bouillon zunahm. Eine etwaige Nachwirkung versprühter Alkohole kann natürlich zu einer Abnahme der Gesamtkeimzahlen führen; Ungenauigkeiten, welche sich nicht vermeiden lassen, wenn man auf nur eine Kammer angewiesen ist. Trotzdem glauben wir, auf Grund

Tabelle 2.

A. Testversuch: Einsaat rund 1000 Staphylokokken im Liter Luft;
(1 Minute vor 0-Zeit): Propylenglykol 1:136000.

Expositionszeit	gezählte Kol.	Summe der aus den Teil- exp. berechn. bzw. z. Z. noch vorh. Keime	in % der Kontrolle
0—60 Min.	2145 2955		
0—5	7475	9057	30.04
5—10	303	1582	5.25
10—15	474	1279	4.24
15—30	617	805	2.67
30—60	188	188	0.62

B. Wie oben:

0—60 Min.	4420 4355		
0—5	810	5859	19.44
5—10	394	1049	3.46
10—15	331	655	2.17
15—30	236	324	1.07
30—60	88	88	0.29

C. Wie oben:

0—60 Min.	13650 12675		
0—5	16770	20345	67.48
5—10	1755	3575	11.86
10—15	715	1820	6.04
15—30	845	1105	3.66
30—60	260	260	0.86

D. Kontrollversuch mit Bakterien allein:

0—60 Min.	20670		
0—5	16900	30141	100.00
5—10	7800	13241	43.92
10—15	3162	5441	18.05
15—30	1200	2279	7.56
30—60	1079	1079	3.58

Temperatur: 20° C.

der mit Alkoholen erzielten Keimverminderungen in unseren Versuchen einigermaßen verwertbare Vergleichsergebnisse erzielt zu haben.

Wir geben im folgenden nur zwei Versuche in extenso wieder, um uns im übrigen auf die Wiedergabe von Wirkungsdiagrammen zu beschränken. Aus Tabelle 2 ist zu ersehen, dass in einer Folge von Testversuchen mit Propylenglykol 1:135000 Volumenteilen Luft zwar recht ungleiche Gesamtkeimzahlen ermittelt wurden, welche zwischen rund 6000 und 20000 auf je drei Liter Luft variierten, während

die Kontrolle etwa 30000 Keime aufwies. Berücksichtigt man aber die relative Verteilung der innerhalb vergleichbarer Zeiträume gewachsenen Kolonien, so zeigt sich in den Versuchen mit Propylenglykol deren gesetzmässige Verminderung. Während im Kontrollversuch 5 Minuten nach Versuchsbeginn noch rund 44 % der Einsaat vorhanden sind, weisen die Propylenglykolreihen nur noch zwischen 12 und 3.5 % Keime auf, nach einer halben Stunde statt 3.6 nur mehr 0.3 bis 0.86 %.

Tabelle 3.

A. Testversuch: Einsaat rund 1000 gelbe Staphylokokken im Liter Luft; (1 Min. vor 0-Zeit): Amylalkohol 1 : 135000.

Expositionszeit	gezählte Kol.	Summe der aus den Teil- exp. berechn. bzw. z. Z. noch vorh. Keime	in % der Kontrolle
0—60 Min.	16640 26780		
0—5	19010	32920	79.82
5—10	5070	13910	33.72
10—15	2834	8840	21.43
15—30	3562	6006	14.56
30—60	2444	2444	5.93

B. Testversuch: Amylalkohol 1 : 67000.

0—60 Min.	4355 1040		
0—5	2405	3172	7.69
5—10	161	767	1.86
10—15	107	606	1.45
15—30	303	499	1.21
30—60	196	196	0.47

C. Kontrollversuch mit Bakterien allein.

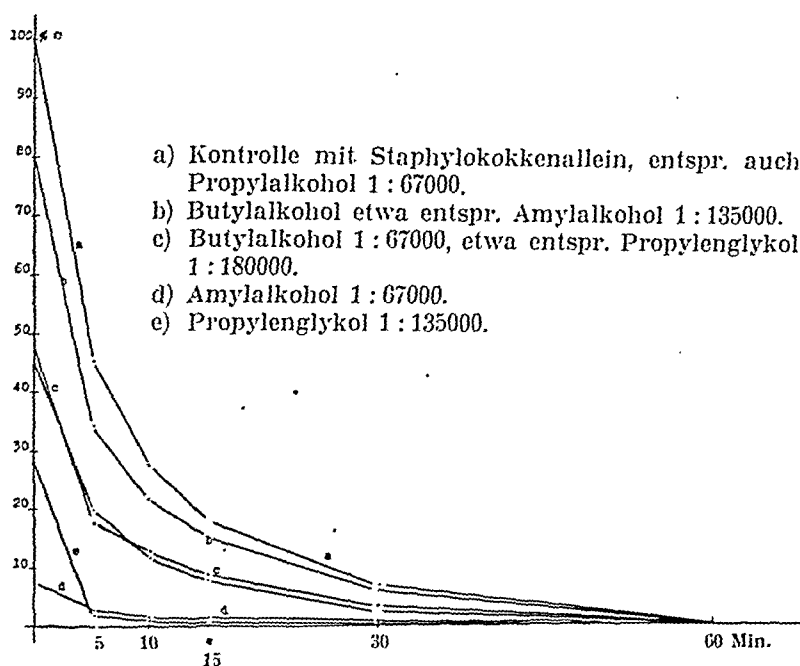
0—60 Min.	32240 24480		
0—5	24155	44250	100.00
5—10	6175	17095	41.43
10—15	2925	10920	26.45
15—30	4615	7995	19.38
30—60	3380	3380	8.21

Temperatur: 22° C.

In Tabelle 3 sind zwei verschiedene Konzentrationen von Amylalkohol u. zw. 1:135000 und 1:67000 Volumenteilen Luft mit dem Kontrollversuch in Vergleich gestellt. Nach 5 Minuten waren nur noch 33.7 bzw. 1.86 % der Keime in den Amylalkoholreihen gegen 41.4 % im Kontrollversuch übrig. Die Ergebnisse sind nicht so in die Augen springend wie nach der Mitteilung von Robertson u. a..

Aus dem Wirkungsdiagramm 2 ist zu entnehmen, dass Propylenglykol in Konzentration 1:135000 nach 5 Minuten nur mehr 1—2 % der Keime gegen fast 45 % des Kontrollversuchs übrig gelassen hat. Bei der Konzentration 1:180000 verschiebt sich dieses Verhältnis nach

5 Minuten schon ungünstig nach etwa 15—25 % gegen 48.5 % der Kontrolle. Bei wärmerer Temperaturlage scheint überdies eine weitere Wirkungseinschränkung stattzuhaben. Propylalkohol weist in den Konzentrationen 1:135000 bis 1:67.000 keine Wirkung im Vergleiche mit der Kontrolle auf. Butylalkohol wirkt erst in der Konzentration 1:67000 merklich, wenn auch nicht entfernt so kräftig wie



Wirkungsdiagramm 2.

Durchschnittliche Wirkungskurven von Propyl-, Butyl- und Amylalkohol im Vergleiche mit den Kurven des Propylenglykol und der Kontrollversuche in bekeimten Atmosphären in geschlossener Kammer. Die Versuche sind aus mehrfachen Einzelversuchen mit den betreffenden Stoffen in je einer oder zwei Dosierungen zusammengestellt. Vergleiche damit Diagramm 3, einen einzeitigen Versuch zum Vergleich der drei Alkohole Butyl- und Amylalkohol und Propylenglykol nebst Kontrolle!

Propylenglykol. Amylalkohol dagegen zeigt sich in der gleichen Konzentration fast so kräftig wie Propylenglykol in der halben.

In Wirkungsdiagramm 3 sind die beiden letztgenannten Alkohole in einer Versuchsserie mit Propylenglykol verglichen und weisen die in zahlreichen Einzelversuchsserien (Wirkungsdiagramm 2) festgestellte Wirkungsfolge: Propylenglykol > Amylalkohol > Butylalkohol > Kontrollversuch in anschaulicher Weise auf. Bei zunehmender Wärme schien die Wirkung aller Stoffe, auch des Propylenglykol, bei den zum Vergleich gebrachten Konzentrationen nivellierend abzunehmen, was in der Zunahme der Flüchtigkeit auch des schwerer flüchtigen Amylalkohol und besonders des Propylenglykol in ungesättigten Atmosphären begründet sein dürfte.

In Tabelle 4 sind den physikalischen Eigenschaften der hier untersuchten Alkohole deren Wirkungen in konzentrierter, flüssiger Form und nach Versprühung gegenübergestellt. Daraus geht hervor, dass die Wirkung der Alkohole *in vitro* in den spezifischen Eigenschaften zu suchen ist, mit welchem jene auf die exponierten Keime zu wirken imstande sind. Vergleicht man aber die Wirkung der versprühten

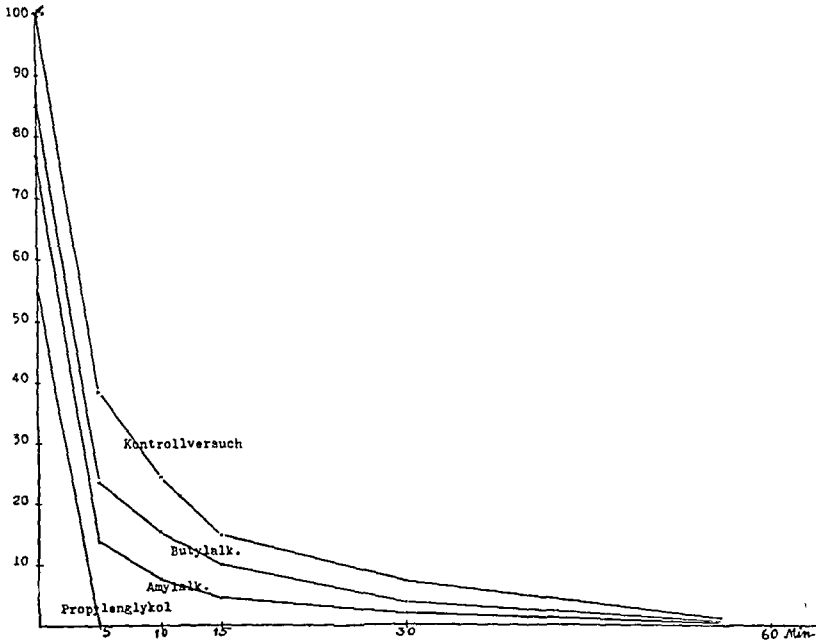


Diagramm 3.

Einzeitiger Versuch zum Vergleich der drei Alkohole Butyl- und Amylalkohol und Propylenglykol nebst Kontrolle. Die Kurven verbinden die Prozentzahlen der in die Versuchskammer eingesprühten Keime, wie sie aus den exponierten Platten während der Versuchszeit nach gewissen unten verzeichneten Zeiten berechnet werden konnten. Die Alkohole waren 1 Minute vor den Keimen in der Kammer versprüht. Die Bakterieneinsaat betrug etwa 7000 gelbe Staphylokokken auf den Liter Luft.

Alkohole in der steigenden Reihe Propylalkohol < Butylalkohol < Amylalkohol < Propylenglykol mit den physikalischen Eigenschaften derselben, so läuft dort die Reihe der Siedepunkte entsprechend $97 < 117 < 130 < 189$ und legt den Schluss nahe, dass die Wirkung der versprühten Alkohole mit den höheren Siedepunkten zunimmt. Dies bestätigt die Annahme Twort's und seiner Mitarbeiter, dass die Wirkung der von ihnen geprüften Lösung von Hexylresorcin in Propylenglykol, welche sich am stärksten keimtötend erwies, zum grossen Teile wenigstens auf der sehr langsamen Verdunstung der feineren Tröpfchen eines derartigen Sprays beruhen müsse. Wenn sie auf Grund ihrer Untersuchungen Wasserlöslichkeit der zur Luftsterilisation versprühten Mittel bei geringer Neigung zur Verflüchtigung for-

Tabelle 4.

Alkohole und deren Eigenschaften:					Wirkung auf Staphylokokken in:	
	spez. Gew.	Dichte	Siedep.	Wasser-lösl.	flüssigem Zustande	bzw. versprühtem Zustande
Propyl-	60.06	0.804	97	unendl.	steril n.) 1 Min.	1:67000 unwirksam.
Butyl-	74.08	0.804	117	7.36	steril n. 1 Min.	1:67000 sehr wenig wirksam.
Amyl-	88.10	0.810	130	2.58	steril n. 4 Min.	1:67000 kräft. wirksam.
Propylen-glykol	76.06	1.040	180	unendl.	steril n. 16 Min.	1:135000 stark wirksam.

dernten und fanden, dass hohe Rideal-Walker-Werte sich bei ihren Versuchen oft als trügerisch erwiesen, so findet auch das in den hier an Alkoholen gefundenen Werten in vitro einerseits und nach Versprühung andererseits seine ungezwungene Bestätigung. Offenbar ist für die Wirkung versprühter Alkohole der Grad der Flüchtigkeit insofern von ausschlaggebender Bedeutung, als hiervon deren Möglichkeit abhängt, mit luftgetragenen Keimen in mehr oder weniger konzentrierter Form zu koagulieren. Die Wirkungsunterschiede der Alkohole bei Einwirkung der flüssigen Aggregate in vitro einerseits, der versprühten Stoffe andererseits gründen also letzten Endes in unterschiedlichen Konzentrations-Wirkungsverhältnissen. Die leicht flüchtigen Alkohole werden bei Versprühung in mehr oder weniger wirkungslose, ungesättigte Gase übergeführt, während die schwerer flüchtigen Amylalkohol und insbesondere Propylen glykol bei nicht zu hoher Temperatur in mehr oder weniger flüssigen Zustände verharren und so, trotz einer gewissen Verdünnung durch Wassercondensation, fast konzentriert zur Wirkung kommen. Dadurch werden die sonst gegebenen Wirkungsverhältnisse der flüssigen Aggregate zugunsten der weniger wirksamen Alkohole verschoben. Die Luftdesinfektion durch Alkohole bietet überdies ein weiteres Beispiel dafür, wie ungeeignet der Phenolcoefficient (Rideal-Walker-Methode) zur Beurteilung von Desinfektionsmitteln ist. Desinfektionsprobleme erfordern in jedem gegebenen Falle die jeweils zweckmässige, methodische Lösung, welche die Eignung eines Mittels sowohl hinsichtlich der in Frage kommenden Schädlinge als auch des Milieus, in welchem diese getroffen werden sollen, zu berücksichtigen hat.

Zu beantworten bliebe noch die Frage, wieweit der Grad der Wasserlöslichkeit, bei sonst gleicher Wirkung in vitro, die Wirkung versprühter Alkohole beeinflusst. Diese Frage sollte sich möglicherweise an Isomeren untersuchen lassen. In besonders innigem Zusam-

menhange mit der Wasserlöslichkeit der fraglichen Stoffe dürfte ihre Lösungsgeschwindigkeit bei der Condensation von Wasserdampf und die relative Feuchtigkeit in geschlossenen Atmosphaeren stehen, wobei mit zunehmender Condensation eine Abnahme, mit abnehmender eine Zunahme der Wirkung zu erwarten, bei abnehmenden Temperaturen aber auch mit gegenteiligen Effekten zu rechnen wäre. Unberührt von diesem Verhalten versprühter Alkohole bleibt ihre Wirkung in gesättigten Atmosphaeren. Überdies ist bezüglich des Propylenglykol noch im Auge zu behalten, dass Versprühung hier möglicherweise diejenigen günstigen Oxydationsbedingungen mit sich führt, welche zur Bildung von Formaldehyd führen. Dies dürfte besonders dann gegeben sein, wenn Glykole nicht nur versprüht, sondern wie in den Versuchen von Stokes und Henle (6) über einer Heizplatte verdampft werden. Ob die Glykole die von diesen Autoren gefundene Wirkung gegen ein mäusepathogenes Influenza-A-Virus auch gegen andere luftgetragene Infekte bewähren werden und die grossen Erwartungen, welche man daher auf sie gesetzt hat, allein werden erfüllen können, bleibt weiterer Untersuchung vorbehalten.

Nach unseren Versuchen bleibt es fraglich, ob die Behandlung luftgetragener, pathogener Keime mit versprühtem Propylenglykol allein zu genügender Abtötung führt. Vergleicht man die zusammengezählten Kolonien aller während kürzerer Zeiten der bekeimten Atmosphaere ausgesetzten Platten mit den Gesamtzahlen der Dauerkontrollen, so sind diese Zahlen zwar nicht gleich, wobei die Dauerexposition besonders im Propylenglykolversuch, weniger bei den anderen Alkoholen und im Kontrollversuch, mehr oder weniger erhebliche Keimverminderung aufweist. Die zusammengerechneten Koloniezahlen der Teilexpositionen aber divergieren lange nicht so stark wie die Zahlen der Dauerexpositionen; ja, sie nehmen im mehrstündigem Versuche entsprechend der in der Keimaufschwemmung einsetzenden Vermehrung zu u. zw. ohne Rücksicht auf die Reihenfolge der Kontroll- und Testversuche d. h. ohne erhebliche Nachwirkung der in der Versuchsfolge etwa angewandten Alkohole. Diese annähernde Übereinstimmung der Gesamtkeimzahlen in Kontroll- und Testversuchen — bei ungleicher prozentueller Verteilung über die Einzelexpositionen — legt nahe, dass ein wesentliches Moment der Glykolwirkung nicht sowohl in der Abtötung als vielmehr in der physikalischen Wirkung einer Niederschlagung der Keime zu suchen sein dürfte, wobei die Atmosphaere schneller ihrer Keime verlustig geht, und diese dann auf dem Boden auf sonstigen Oberflächen im Raume mit den fallenden Tröpfchen niedergeschlagen werden, um dort erst allmählich abzusterben. Es bleibt daher noch nachzuweisen, ob in der Praxis von dem reinen Propylenglykol ein ausreichender Desinfektionseffekt zu erwarten steht.

Zusammenfassung.

Bei ihren Untersuchungen über die Versprühung von Mitteln, welche in der Luft verteilte Keime unschädlich machen, fanden Twort u. a. Phenole in Propylenglykol als Vehikel besonders geeignet. Die Autoren folgerten aus ihren eingehenden Versuchen, dass wasserlösliche und nicht allzu flüchtige Stoffe oder Mischungen der genannten Art, die nach der Versprühung eine gewisse Beständigkeit der Teilchen in der Luft aufweisen, am besten geeignet sein müssten, dem angestrebten Zwecke zu dienen.

Wir haben an einer Gruppe chemisch reiner Stoffe diese Frage nachgeprüft u. zw. an den niederen Alkoholen Propyl-, Butyl- und Amylalkohol im Vergleich mit dem zweibasigen Propylenglykol. Wir fanden, dass — ganz unabhängig von der Wirkung der reinen, flüssigen Substanzen in vitro, bei deren Prüfung sich die Reihenfolge Aethylalkohol < Propylenglykol < Amylalkohol < Propylalkohol < Butylalkohol ergab, — die Versprühung der genannten Substanzen die unerwartete Reihenfolge Propylenglykol > Amylalkohol > Butylalkohol > Propylalkohol ergab, eine Reihe, welche zunehmender Flüchtigkeit der genannten Stoffe entspricht.

Man muss also annehmen, dass die Wirkung dieser Stoffe bei der Versprühung — in Ermangelung der Erzielung mehr oder weniger gesättigter Atmosphären flüchtiger Körper, deren den reinen flüssigen Substanzen vermutlich analoge Wirkung noch zu prüfen wäre — davon abhängt, wieweit sie in mehr oder weniger konzentrierter Form, abhängig von Lufttemperatur, -druck und -feuchtigkeit, mit den schwebenden Keimen koagulieren können. Dies ist in erster Linie von der Flüchtigkeit (Siedepunkt) und der Wasserlöslichkeit der Alkohole abhängig.

Wir konnten also die Annahme Twort's und seiner Mitarbeiter für die reinen Alkohole im wesentlichen bestätigen. Darüber hinaus geht aus unseren Versuchen hervor, dass die Wirkung des reinen, versprühten Propylenglykol zu einem wesentlichen Teile eine physikalische d. h. fällende sein muss, nur in begrenztem Masse eine abtötende sein kann. Ob daher eine allgemeinere, durchgreifende Luftdesinfektion, wie sie Stokes und Henle für das Influenzavirus beschreiben, von Propylenglykol allein zu erwarten ist, müssen weitere Versuche zeigen.

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Abgeschlossen den 18. Juni 1944.
Stockholm, Barnhusgatan 4, IV.

STUDIES ON HEREDITARY DWARFISM IN MICE. VI.*)

ANATOMY, HISTOLOGY AND DEVELOPMENT OF THE PITUITARY AT HEREDITARY ANTERIOR PITUITARY DWARFISM IN MICE

By *Torben Francis*, M.D.

(Received for publication October 22nd, 1944).

1. Introduction. Technique.
2. Anatomy.
3. Histology. a. normal mice. b. dwarf mice.
4. Development. Histogenesis of the anterior lobe of the dwarf mouse.
5. Hypothesis as to the cell cycle.

1. Introduction.

The strain of black-silver mice in which *Snell* (1929) found hereditary recessive dwarfism had in 1928 been brought from England to the U. S. A. *Snell* (1930) found the thyroid gland of the dwarfs to be under-developed. By treatment with thyrotropic hormone and by treatment with thyroid tissue the dwarfs grew considerably in size, so accordingly it seemed likely that a thyroid insufficiency should be the cause of the dwarf growth. But in 1930 *Smith & MacDowell* showed that also the anterior pituitary lobe, the sexual glands, and the adrenal cortex are underdeveloped, that the dwarf mice by treatment with transplantation of normal rat pituitaries grow up to become almost normal animals, and that the adrenals, the thyroid, and the sexual glands of the treated animals develop into an almost normal size and appearance. This seems to be indicative that the primary cause of dwarfism should lie in the pituitary — and more exactly in the anterior pituitary lobe.

*) Previous publication in this series: *Acta path. et microbiol. Scand.* XIII, 512, 36 — XIV, 197, 37 — Suppl. XXXVII, 290, 38 — XVIII, 20, 41 — XVIII, 169, 41 — XIX, 563, 42.

For further particulars see the author's monograph: *Investigations into the development of the pituitary at hereditary anterior pituitary dwarfism in mice with reference to the pathogenesis of the anterior pituitary dwarfism.* pp. 154. Munksgaard. Copenhagen. 1944.

To Miss P. A. Brandt's Legacy for the Promotion of Pathologic-physiological Research and Physiologic-gymnastic Science and to the P. Carl Petersen Funds I am indebted for financial support.

Thus it is evident that the anterior pituitary dwarf mice constitute a rare and unique animal material, not only for experimental investigations into the function of the anterior pituitary lobe as a superior centre of the process of growth, but also an extremely valuable material as a basis for investigations into the effect of the hormones of the endocrine glands on the organism. In realisation of this fact a number of examinations have been made of the anatomy, the physiology, and the pathology of the dwarfs, and besides, thorough investigations into the reaction of the dwarfs to treatment with hormones from the anterior lobe and the endocrine glands inferior to the anterior lobe. Most of these investigations have been made in the U. S. A. (*Smith & Mac Dowell, Mac Dowell & Laanes, Bates, Laanes & Riddle, Dawson, Osborn, Osborn & Boettiger, and Marshak*). But thorough investigations with special reference to the growth, the histology of the endocrine glands, and the reaction of the dwarfs to treatment with hormones have specially been carried out in Copenhagen, since *Kemp* in 1933 brought 3 black-silver mice of the strain that is heterozygous for dwarfism from *Mac Dowell's* laboratory in the Department of Genetics of the Carnegie Institution of Washington to the University Institute for General Pathology of Copenhagen, where he continued the breeding. Later, after the establishment in 1938 of the University Institute for Human Genetics, the strain of dwarf mice was transferred to this Institute.

Smith & Mac Dowell (1930) were the first to demonstrate that the anterior pituitary lobe of dwarfs is markedly hypoplastic, whereas the intermediate and posterior lobes are normal.

According to *Smith & Mac Dowell* the anterior lobe is transformed into a network of more or less compact connective tissue. Eosinophiles are lacking completely. As to the presence of basophiles *Smith & Mac Dowell* are reserved. By staining (Altmann's acid fuchsin and acid violet according to Bailey's technique, Mallory's connective-tissue stain, and Masson's trichrome stain) no basophile cells could be seen in the anterior lobe. As, however, there was found no satisfactory impregnation of the basophiles in the normal control mice either whereas it was found in the anterior lobe of other animals) *Smith & Mac Dowell* have left the problem unsolved. The chromophobe cells are much reduced in number.

Kemp & Marx (1937) found in the anterior lobe the same changes as *Smith & Mac Dowell*, but besides the transformation of the greater part of the anterior lobe into a connective-tissue network they also observed in the organ places containing a great number of epithelial cells of irregular shape without granulations. Only occasionally in some very few preparations did they find eosinophile cells (most often in the periphery of the anterior lobe and generally in rather old dwarfs treated with hormones). Also a few basophile cells could be seen in the anterior lobe of the dwarfs, but of a different type. Whe-

ther the number of basophiles differs from that found in normal animals could not be decided on, the number of basophiles being also in the normal animals very small. According to *Kemp & Marx* the hypoplasia of the anterior lobe is particularly pronounced corresponding to the medial area making the »floor« of the pituitary cavity, which may be transformed into a very thin membrane of connective-tissue cells, whereas the lateral parts of the anterior lobe, which in some cases are markedly assymetrical, may be more or less well developed.

The dwarf growth does not manifest it self till 12 to 14 days after birth.

Technique. The pituitaries of 42 normal mice, 44 dwarf mice and 200 fetuses and young of the dwarf strain ranging in age from 9 fetal days till 12 days after birth have been submitted to investigation. All the fetuses and young were born of »dwarf parents«, i. e. male and female mice in whose previous offspring there occur dwarfs, so that the parents are known to be heterozygous for anterior pituitary dwarfism. And in the material of fetuses and young until the age of 12 days only complete litters are included, i. e. the total number of living fetuses and young of each litter, so as to avoid in the material a selection that might cause relative changes in the numbers of dwarfs and normals.

The age of the fetuses was known before the excision of the uterus, as before the copulation vaginal smears had been taken daily from the maternal parents, and the female mice being in proestrus or estrus had been placed in a cage with a male mouse from 3 to 12 hours. If there has been copulation a »vaginal plug« is seen the following day in the vagina of the female mouse. The vaginal cycle ceases if fecundation has taken place, and 11 days after the fecundation blood-filled viscid matter appears in the vagina as a certain sign of pregnancy.

Fixation: Helly's fluid. Orths fluid. 10 per cent formol.

Staining: Hematoxylin-eosin. Hematoxylin-Romanowsky-Giemsa-azureosin. Iron-hematoxylin after Regaud-Heidenhain. Mallory's connective tissue staining.

2. *Anatomy.*

The shape and size of the pituitary (figs. 5—8) varies in the dwarfs far more than in the normals, but when the dwarf has passed a certain age there is always found marked hypoplasia of the anterior lobe, perceptible already on the macroscopical examination of the pituitary. The length of the pituitary body varies from $\frac{1}{2}$ to 1 mm, the breadth from 1 to $1\frac{3}{4}$ mm. The anterior lobe hypoplasia brings about partly a flattening of the whole pituitary, partly a shortening of the dimensions in the antero-posterior direction, it being, however, mainly the lateral portions that are small and hypoplastic. The medial portion of the pituitary, comprising the pars intermedia, the neurohypophysis, the infundibulum, and the pituitary cavity, is comparatively well-preserved and not diminished more than what is due to the general retardation of growth; and the pituitary cavity is only slightly smaller and flatter than that of normal animals.

3. *Histology.*

a. Normal mice.

The two main groups of cells in the anterior lobe of normal mouse are the chromophobes and the acidophiles, whereas basophiles occur but rarely.

Chromophobe Cells.

1. *Typical chromophobe cells* are 4 or 5 my in size, round, oval, or angular with indistinct cell outlines. The nuclei are round or oval, containing a medium amount of chromatin, 2 or 3 my in diameter, or larger and poor in chromatin, situated in the centre of the cell or slightly excentrically with 1 or 2 acidophilic nucleoli. The cytoplasm is scanty, unstained (hematoxylin-eosin), yellowish grey (Regaud-Heidenhain), or faintly bluish (Mallory). A Golgi zone or vacuoles can only very rarely be observed. There are found no granulations. The typical chromophobe cells constitute about 40 per cent of the anterior lobe cells both in young and older mice and are found evenly distributed all over the anterior lobe.

2. *Small chromophobe cells with a pyknotic nucleus (small pyknotic chromophobe cells)* are chromophobes with a very scanty, hardly visible cytoplasm. They lie single or closely packed in small groups and are 2 or 3 my in size, flattened or wedge-shaped with an irregular, flat nucleus rich in chromatin. The cytoplasm is unstained, and there are found neither Golgi zone, vacuoles, nor granulations. They occur scattered all over the anterior lobe and constitute never more than 3 to 6 per cent of the anterior lobe cells.

Acidophile Cells.

The acidophiles constitute 45 to 55 per cent of the anterior lobe cells thus being the predominant cell element. They are found distributed all over the anterior lobe, but are particularly numerous and densely packed in the periphery. The number decreases somewhat with advancing years. They occur in the following forms:

1. *Hypoacidophile cells* are 5 to 7 my in diameter, somewhat angular, flattened or notched. The nucleus is round or oval with a medium amount of chromatin. The cytoplasm is faintly acidophilic and contains but few granulations. In some cases there are found small vacuoles and sometimes a Golgi zone.

2. *Typical acidophile cells* (figs. 9, 10, 11) are 6 to 8 my in diameter, round, oval, or angular, with distinct cell outlines. The nuclei are round or oval, 3 or 4 my in diameter with distinct chromatinic grains and 1 or 2 acidophilic nucleoli generally located centrally. The abundance of cytoplasm is intensely acidophilic with granulations,

which, however, by staining with eosin are rather indistinct, but by staining with iron hematoxylin (fig. 11) stand out clearly as small, closely packed, fine granulations, which may cover completely the other cell elements. In the vicinity of the nucleus there is seen a more or less marked Golgi zone appearing either as a clear ring round the nucleus (fig. 10) or as a round distinct clear space on one side of the nucleus (fig. 9). Scattered about the cytoplasm there are found a smaller or greater number of vacuoles of different sizes, and in some cases the cell is quite filled with small vacuoles.

3. *Large acidophile cells* (fig. 12) are typical acidophiles with round or oval nuclei and distinct cell outlines. They may be up to 12 to 14 my in diameter. The cytoplasm is acidophilic with numerous granulations and often a number of small vacuoles, whereas the Golgi zone is generally absent or, if present, indistinct. The cytoplasm is abundant and the cells most frequently round (secretion-filled).

4. *Hyperacidophile cells* are somewhat smaller than the typical acidophiles with a diameter of 4 or 5 my. The cytoplasm is intensely acidophilic with closely packed, rather indistinct granulations, and there can be seen no vacuoles and no Golgi zone. The nucleus is round or oval and rich in chromatin.

5. *Small acidophile cells with a pyknotic nucleus (small pyknotic acidophile cells)* have a very scanty, faintly acidophilic cytoplasm. They are flat, angular, or wedge-shaped, 2 or 3 my in size with indistinct or quite invisible cell outlines. The nucleus is pyknotic, small, reniform or bean-shaped, or quite flattened and notched, very rich in chromatin with compact structures. The cytoplasm is extremely scanty, homogeneous, faintly acidophilic, containing neither granulations, Golgi zone, nor vacuoles. The cells lie either single, 2 or 3 together, or in small groups (islands), arranged as in glandular acini with up to 4 to 6 cells in each group.

Basophilic Cells.

Basophiles are generally not found in the anterior pituitary lobe of mice. When they do occur it is nearly always in mice of advanced age and in pregnant females. They may vary somewhat in shape and size ranging from 3 to 6 my in diameter. The cytoplasm is rather scanty, distinctly basophilic with densely packed basophilic granulations (fig. 13). Sometimes there is found an indistinct Golgi zone, but generally no vacuoles. The nucleus is round with a medium amount of chromatin and centrally located. These cells lie scattered and single — never gathered in groups — and if they do occur they constitute less than 1 per cent of the total number of glandular cells in the anterior lobe.

Examples of the proportion of the anterior lobe cells in the typical normal mouse and the dwarf mouse.

	Normal mice		Dwarf mice			
Age in days	25	219	13	24	41	273
No.	240	274	201	234	249	275
Typical chromophobes	++	++	+	+	+	(+)
Hypoacidophiles	(+)	(+)	(+)	o	(+)	o
Typical acidophiles	++	++++	+	o	(+)	o
Large acidophiles	++	(+)	(+)	o	o	o
Hyperacidophiles	(+)	(+)	(+)	(+)	(+)	(+)
Small pycnotic acidophiles	(+)	(+)	++	++++	++++	++++
Small pycnotic chromophobes	(+)	(+)	++	++++	++++	++++
Basophiles	(+)	o	o	o	o	o

The number of cells of the different cell types are indicated according to the following principle:

- ++++ the total number
- +++ the great majority
- ++ numerous
- ++ many
- + some (scattered in the field of vision)
- (+) a few
- o none

Other Elements in the Anterior Pituitary Lobe.

1. *Pregnancy cells.* In the anterior lobe of the pregnant female mouse there may occur, besides the basophiles, which are found almost invariably, special pregnancy cells, which are large, round basophile cells with the nucleus lying excentrically pressed against the cell wall by an enormous colloid-containing vacuole. The cytoplasm lies in a ring round this vacuole. Thus the whole cell gets the shape of a signet-ring (fig. 14).

2. *Colloid* is found in the anterior lobe either in the form of cysts or, more rarely, lying between the cells. Colloid cysts are generally found in mice of advanced age, but may also be observed in immature mice from 5½ to 21 days of age. Such cysts are round or oval lined with a flat or cuboid epithelium and having a faintly acidophilic colloid content, in which there may often be seen desquamated epithelial cells (fig. 15). In the young animals the colloid cysts are very small, in the older ones somewhat larger.

3. *Vascular supply.* The entire anterior lobe is richly vascularized and filled with elastic sinuous vessels, particularly so in the large lateral portions. The red blood corpuscles are found in rouleaux for-

mations in sinuses between the glandular acini. Also in the connective tissue capsule there are seen numerous blood vassels.

4. *Connective tissue.* The glandular acini are separated from each other by fine connective tissue septa, and besides there proceed from the capsule some somewhat coarser connective tissue bands down through the anterior lobe. Like the connective tissue capsule also the connective tissue of the anterior lobe originates from the vascular embryonal connective tissue in the base of the skull, which at an early stage of the embryonic period has been covered by the anterior lobe (see later). The connective tissue capsule of the pituitary, which is loose and easily removable, passes upwards at the infundibulum to become united with the dura mater.

b. Dwarf mice.

When the anterior lobe of a dwarf mouse is studied in the microscope under low power magnification the most conspicuous and peculiar features of the picture are, besides the hypoplasia and deformity of the lobe, the extraordinary abundance and density of cells and the compact structure of the tissue. On a closer inspection under high power magnification (fig. 16) the condensed and compact tissue proves to consist of small, angular or flattened, closely packed, chromophobe and acidophile epithelial cells; and by a systematic review of preparations cut in serial sections of the anterior lobe of dwarfs it appears that the glandular epithelial cells and the other anterior lobe elements occur in the following forms:

Chromophobe Cells.

1. *Typical chromophobe cells* are found in a comparatively great number in quite young dwarfs, whereas the number is greatly reduced in the full-grown dwarfs.

2. *Small chromophobe cells with a pyknotic nucleus (small pyknotic chromophobe cells).* Their number is considerably increased both in young and old dwarfs (about 40 per cent of the anterior lobe cells). Together with the small pyknotic acidophiles these cells constitute the preponderating and predominant part of the anterior lobe parenchyma. They are found scattered throughout the entire anterior lobe or in islands, often located together with small pyknotic acidophiles.

Acidophile Cells.

Within the group of acidophiles the following types can be distinguished:

1. *Hypoacidophile cells* are completely absent in dwarfs of advanced age. In younger animals they are found only in a very small number, and sometimes they are even totally absent.

2. *Typical acidophile cells* are in young dwarfs found single or in small groups generally in the periphery. Their number is always very small, and when the dwarfs have reached the age of seven weeks they are completely absent.

3. *Large acidophile cells* are not present.

4. *Hyperacidophile cells*. Their number varies a great deal. Most frequently it is slightly increased, especially in the dwarfs of advanced age and in the very young dwarfs.

5. *Small acidophile cells with a pyknotic nucleus (small pyknotic acidophile cells)*. Their number is exceedingly increased (40 to 50 per cent of the anterior lobe cells). The small pyknotic acidophiles together with small pyknotic chromophobes predominate the whole anterior lobe picture, so that one has to search intensely in order to find the other cells. Except for the faintly acidophile cytoplasm they resemble completely the small pyknotic chromophobe cells, with which they lie in intimate relation either scattered about the whole anterior lobe or arranged in islands (figs. 16, 17).

Basophile Cells.

Basophile cells do not occur in the anterior pituitary lobe of the dwarfs.

Other Elements in the Anterior Pituitary Lobe of Dwarf Mice.

1. *Colloid* is not found extracellularly in the anterior lobe of the dwarf, and colloid cysts are not seen, neither in young nor in old dwarfs.

2. *Vascular supply*. The anterior pituitary lobe of the dwarf is vascularized to the same extent as that of the normal mouse. The vessels contain a little less blood, which is due to the fact that the glandular tissue is so firm and compact that it does not permit of so great a distention of the vessels as that of the normal lobe.

3. *Connective tissue*. There is no increase in the interfillicular and intercellular connective tissue, and there is seen no ingrowth of connective tissue in the anterior lobe, nor any connective-tissue-like transformation of the glandular parenchyma. But, as mentioned by Kemp & Marx (1937) the floor of the pituitary cavity may be so thin that only the connective tissue capsule remains in this place. The connective tissue capsule of the dwarf pituitary is besides thinner and finer than that of the normal pituitary.

The pars intermedia (intermediate lobe) in the normal mouse, consists of several rows of cylindric epithelium of slender closely packed cells. The cells are uniform and do not stain with eosin. There

are seen no vessels in the intermediate lobe, nor are there any signs of a colloid production. In the dwarf mouse the intermediate lobe is not subject to hypoplasia or cellular degeneration. It is somewhat smaller than the intermediate lobe of the normal mouse in consequence of the smaller size of the animal, but otherwise it seems to be quite normal.

The pars infundibularis and *the pars tuberalis* consists in the normal mouse of chromophobes and acidophiles that are somewhat smaller than the corresponding cells in the anterior lobe. In the dwarf mouse these parts of the adenohypophysis present cellular changes corresponding to those seen in the *pars glandularis*.

The neurohypophysis consists in the normal mouse of spindle-shaped nerve cells and glia cells. It is richly vascularized and the vessels are often seen to contain abundant acidophilic colloid. There are found no basophile cells, no intracellular colloid, and no cysts. The neurohypophysis of the dwarf is somewhat smaller than that of the normal mouse, but otherwise it corresponds exactly to the latter.

The infundibulum presents no abnormal features in the dwarf mouse.

4. Development.

On the 9th fetal day the anlage of the adenohypophysis is developed as a small epithelial evagination on the foregut. At first it is plate-shaped and consists of one or two rows of cylindric epithelium with basally located cell nuclei. The cells are embryonal epithelial cells of simple construction with scanty cytoplasm and the nuclei are rich in chromatin with a small acidophilic nucleolus. From the stomodeum the adenohypophysis anlage projects into the loose connective tissue at the base of the skull in the direction towards the floor of the anterior cerebral vesicle.

On the 11th fetal day (figs. 18, 19) the adenohypophysis has developed into a deep epithelial pouch with 3 rows of cylindric epithelium, the cells of which have long nuclei rich in chromatin, many mitoses, and a scanty amount of cytoplasm. At the more advanced stages the adenohypophysis is constricted off entirely or in part from its connection with the stomodeum being now an independent epithelial vesicle in the connective tissue of the base of the skull.

On the 12th fetal day the adenohypophysis has lost every epithelial connection with the stomodeal epithelium.

Between the 14th and the 15th fetal day the anterior lobe is transformed from a primitively constructed epithelial organ to an endocrine gland with a specific glandular epithelium. Already on the 14th day a few small, faintly acidophile cells (hypoacidophiles) may be seen here and there. Between the 14th and the 15th fetal day the

anterior lobe anlage suddenly grows considerably in size through a greatly increased cell proliferation. By this process there are formed on the anterior surface large epithelial ridges and edges separated from each other by deep notches. The epithelial edges fold over the vessels of the fetal connective tissue in the base of the skull. The epithelial cells in the anterior lobe place themselves close to the vessels lined with endothelium; they even enclose the vessels so that the latter get to lie in the anterior lobe itself. Horizontal sections show that by this process the cell trabecles of the anterior lobe become radially arranged.

Simultaneously there occurs an essential change in the composition of the anterior lobe parenchyma: The small embryonal epithelial cells with scanty cytoplasm and densely packed nuclei are transformed into round or polygonal or cuboid cells with well-defined cell outlines, and the cells arrange themselves into trabecles and acini as they are seen in the normal adult animal. The cytoplasm of by far the greater number of cells is ungranulated, unstained, and structureless (chromophobes), but besides there are found many cells with a slightly granulated acidophilic cytoplasm (typical acidophiles). Moreover there are seen some odd hypoacidophiles, hyperacidophiles, small pyknotic acidophiles, and small pyknotic chromophobes.

By this time the pars infundibularis has grown out as a crest-like epithelial ridge from the anterior surface of the anterior lobe anlage, from where it points towards the floor of the 3rd ventricle. It consists of small still but slightly differentiated epithelial cells with a non-staining cytoplasm. The neurohypophysis is by this time hemispherical in shape with a thin canalicular lumen. In the wall there is seen proliferation of small embryonal epithelial cells.

At *bith*, which occurs on the 22nd fetal day, the histological picture of the anterior lobe changes but very little. The granulations become somewhat more well-defined and more numerous and closely packed in the typical acidophiles, and in many of these cells there is now seen a distinct Golgi zone lying either direct on the nucleus or forming a more or less complete ring about the latter. The shape of the anterior lobe varies a great deal. Most often it is short and rounded, or more slender and spindle-shaped. In sagittal section the pituitary is approximately of the same shape as that of the adult mouse, though not quite so flattened as the latter.

5 and 6 days after birth a few of the young present marked hypoplasia of the anterior lobe. The hypoplastic anterior lobe is thin, slender, and more sharp-edged than the normal one, and in some cases even somewhat assymmetrical (figs. 20, 21). In the normal anterior lobes the number of typical acidophile cells increase somewhat and that of the typical chromophobe cells decreases correspondingly.

8 days after birth large acidophile cells are regularly seen scattered in the periphery of the anterior lobe.

9 days after birth there is found a narrow Golgi zone in a great number of the typical acidophiles. One single anterior lobe appears to be slightly hypoplastic.

12 days after birth 2 out of 4 anterior lobes are subject to marked hypoplasia.

Histogenesis of the Anterior of the Dwarf Mouse.

The changes characteristic of the dwarf in the anterior lobe parenchyma are as described: a. A great increase in the number of small pyknotic acidophiles and chromophobes. b. Complete absence or a greatly reduced number of typical acidophiles and chromophobes. c. Complete absence of large acidophiles and a greatly reduced number of hypoacidophiles. The number of hyperacidophiles is generally slightly increased. d. Density of the glandular tissue.

By a systematic study of the anterior lobe parenchyma in 200 examined fetuses and young of the dwarf strain the observation has been made, that in 31 fetuses and young between the ages of 20 fetal days and 12 days after birth the anterior lobe presents a glandular parenchyma corresponding in a more or less pronounced degree to the picture characteristic of the anterior pituitary lobe of the dwarf mouse.

In its lightest form this change deviating from the norm consists merely in a slight increase in the number of small pyknotic acidophiles and small pyknotic chromophobes. The small pyknotic cells lie, like in normal animals, in small »islands« with 4 to 7 cells in each, and the difference consists in a slight increase in the number of such »islands«. This slight increase in the number of small pyknotic cells is found in 9 fetuses and young between the ages of 20 fetal days and 9 days after birth.

In 16 of the 31 fetuses and young there is found in the anterior lobe, besides the increase in the number of small pyknotic cells and in some cases also of hyperacidophiles, also a more or less pronounced reduction in the number of typical acidophiles.

In 6 of the 31 fetuses and young, which are from 6 to 12 days of age, thus being among the oldest of the immature mice in the material, there is seen in the anterior lobe a very considerable increase in the number of small pyknotic cells in connection with a marked reduction in the number of typical acidophiles and chromophobes, so that there can be no doubt that these 6 anterior lobes are typical dwarf anterior lobes.

Conclusion.

1) In dwarfs as well as in non-dwarfs the anlage of the adeno-hypophysis develops on the 9th fetal day and that of the neuro-hypophysis on the 11th fetal day.

2) Until the 14th fetal day the adenohipophysis consists of fetal epithelial cells poor in cytoplasm. Between the 14th and the 15th fetal day the anterior wall of the adenohipophysis (the anterior lobe) is transformed, through cell proliferation, folding over vascular connective tissue in the base of the skull, and cell differentiation, from being part of an epithelial vesicle consisting of primitive fetal cells into a glandular organ containing connective tissue and blood vessels, and with a specific glandular parenchyma.

3) The homozygous gene for dwarfism manifests itself in the anterior lobe of the dwarf at some time or other between the 20th fetal day and the 12th day after birth by an increase in the relative number of small pyknotic cells and a reduction in that of the typical acidophiles.

4) This change is probably preceded by a stage in which only a slight increase in the number of small pyknotic cells can be observed.

5) Until 5 days after birth the formation and growth of the pituitary has a fairly uniform, parallel, and normal course in dwarfs and non-dwarfs.

6) Hypoplasia of the anterior lobe is observed in some of the dwarfs from the 5th day after birth. The hypoplasia is slightest on the 5th and the 6th day after which it gradually increases. On the 12th day it may be very pronounced. It is seen only in the dwarfs whose anterior lobe is subject to pronounced parenchyma changes and must accordingly be regarded as secondary in relation to such changes.

5. Hypothesis as to cell cycle.

A schematic setting up of a cell cycle in the anterior lobe can of course — as pointed out by *Franck* — be no more than a mere hypothetical one, but it may serve to give an idea of the differences in cellular development between the anterior lobes of normals and dwarfs that in the dwarfs lead to hypoplasia and deformation of the anterior lobe thus conditioning the dwarf growth.

Fig. 1 gives a schematic presentation of the author's hypothesis as to the cycle in the anterior lobe of *the normal mouse*:

The typical chromophobe cell or mother cell (A) is first transformed into a hypoacidophile (B), which is the first cell to occur in the anterior lobe of the fetus after the formation of the typical chromophobes. From the hypoacidophile the typical acidophile (C) is developed, the latter being not found till after the presence of the former has been demonstrated (the 15th fetal day).

The typical acidophile may a) fill with secretion and develop into a large acidophile (D) or b) liberate its secretion and develop into a hyperacidophile (E): possibly it may also c) degenerate direct into

a small pyknotic acidophile (F); yet another possibility is d) that it is transformed into a basophile (G).

The large acidophile (D) may by liberation of some of its secretion redevelop into a typical acidophile (C), or by liberation of the greater part of its secretion be transformed into a hyperacidophile (E).

The hyperacidophile (E) may by renewed hormone production redevelop into a typical acidophile (C) and thus again take part in the cycle, or it may by further draining off be transformed into a small pyknotic acidophile (F).

The small pyknotic acidophile (F) is an acidophile cell with a minimum of function. In by far the greater number of cases it is transformed into a small pyknotic chromophobe (H), which is the final stage in the cycle. But it may possibly in a few cases redevelop into a typical acidophile and thus again take part in the cycle.

The basophile (G) does not take a regular part in the cycle. Probably it is developed from the typical acidophile (C) like the hyperacidophile, which it resembles a great deal both in structure and size, and presumably it may redevelop into a typical acidophile.

By observing the anterior lobe of the *dwarf* in the microscope one gets the impression of an extremely pronounced parenchymatous defect. The hormone production, if at all present, seems to be so insignificant that it can hardly assert itself sufficiently to distinguish the dwarf from the hypophysectomized mouse, because in the adult dwarf there is complete absence of the anterior lobe cells that, in the opinion of most investigators and according to the hypothesis advanced here as to the cycle in the anterior lobe of the normal mouse, are the proper hormone-producing cells, viz. the typical acidophiles.

If on the other hand it is presumed, as in the hypothesis, that the hyperacidophiles have some and the small pyknotic acidophiles a very slight power of producing hormone, the histological findings in the anterior lobe bear out the observations suggesting that the hormone production of the dwarf is not completely stopped.

Figs. 2—4 show the author's hypothesis as to the cycle in the anterior lobe of the dwarf at its different developmental stages:

At the first stage, between the 20th fetal day and the 9th day after birth, the cellular changes consists simply in an increase in the number of small pyknotic cells (fig. 2): The typical acidophile (C) is like that of the normal anterior lobe developed from the hypoacidophile (B), but its life is shorter than normally, and many of the typical acidophiles are abnormally soon transformed either direct or by the hyperacidophiles (E) into small pyknotic acidophiles (F), and from them further into small pyknotic chromophobes (H).

At the ensuing second stage the number of small pyknotic cells is increased and that of typical acidophiles decreased, and there are seen slight proportional changes in the numbers of typical chromo-

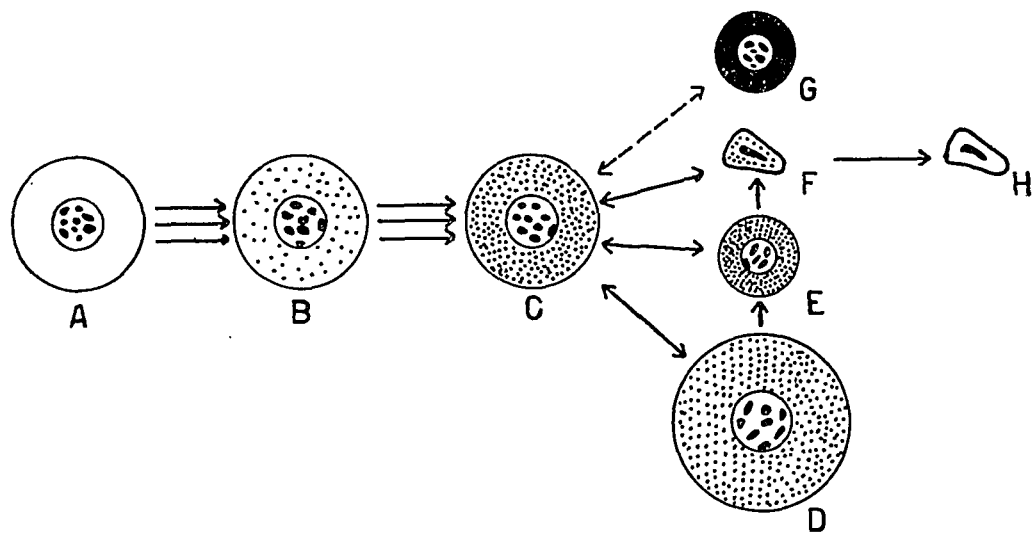


Fig. 1.

Cycle in the anterior pituitary lobe of normal mice.

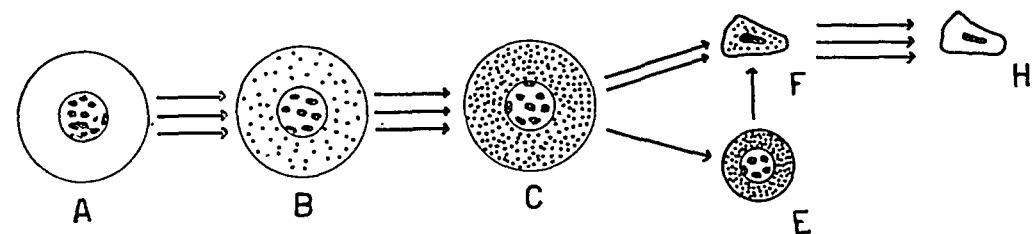


Fig. 2.

Cycle in the anterior pituitary lobe of very young dwarfs. Stage I.

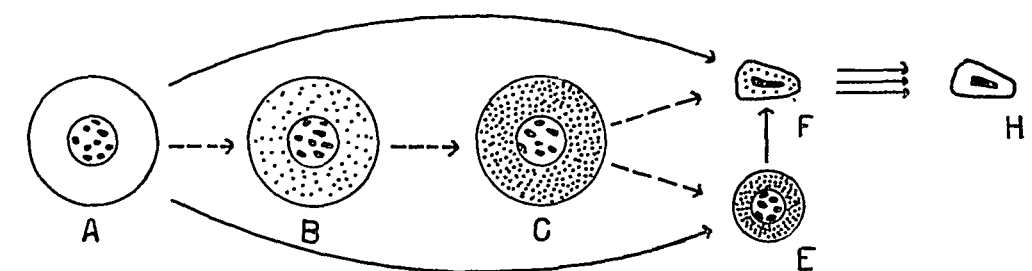


Fig. 3.

Cycle in the anterior pituitary lobe of young dwarfs. Stage II.

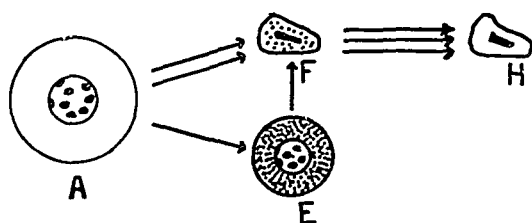


Fig. 4.

Cycle in the anterior pituitary lobe of dwarfs over 6-7 weeks of age. Stage III.

A typical chromophobe
 B typical acidophile
 C typical acidophile
 D large acidophile
 E hyperacidophile
 F small pyknotic acidophile
 G basophile
 H small pyknotic chromophobe

A typical chromophobe
 B typical acidophile
 C typical acidophile
 D large acidophile
 E hyperacidophile
 F small pyknotic acidophile
 G basophile
 H small pyknotic chromophobe

phobes, hyperacidophiles, and large acidophiles (fig. 3): Numerous typical chromophobes (A) are transformed direct or by the hyperacidophile stage (E) into small pyknotic acidophiles (F), and only a certain number pass the normal way by the hypoacidophiles (B) and the typical acidophiles (C).

At the third stage, which may in a few cases occur as early as the 20th day after birth, and which has always occurred by the 47th day, the typical acidophiles and the hypoacidophiles are completely lacking. The anterior lobe consists exclusively of some few typical chromophobes, a somewhat varying number of hyperacidophiles, and numerous small pyknotic acidophiles and chromophobes and the cycle in the anterior lobe of the dwarf mouse has got »a proportional change to the right« (fig. 4): All the typical chromophobes (A), the number of which is greatly reduced, are most frequently transformed direct, but also occasionally by the hyperacidophile stage (E) into small pyknotic acidophiles (F) and small pyknotic chromophobes (H).

The transformation of the typical chromophobe cells direct into hyperacidophiles and small pyknotic acidophiles with a following »proportional change to the right« in the anterior pituitary of the dwarf mouse means in other words, that the nucleus of the glandular cell in the anterior pituitary is getting pyknotic i. e. begins to degenerate in the same moment, in which the cytoplasm of the cell begins to become acidophile i. e. the cell begins to work as a hormone-producing glandular cell.

Summary.

42 normal mice, 44 dwarf mice and 200 fetuses and young of the dwarf strain have been investigated with reference to the anatomy, the histology and development of the anterior pituitary of the dwarf mouse.

There is no connective tissue-like transformation of the dwarf's anterior lobe tissue nor ingrowth of connective tissue — as suggested by previous investigations.

The anterior pituitary lobe of the dwarf mouse consists of numerous small pyknotic acidophiles and small pyknotic chromophobes completely dominating the anterior lobe picture. The number of typical chromophobe cells is greatly reduced. In young dwarfs there are found a few hyperacidophiles and typical acidophiles. When the dwarf is more than 6 or 7 weeks old they are completely absent. The number of hyperacidophiles is varying, most often it is somewhat increased. There are no basophiles and no intercellular colloid or colloid cysts.

The parenchymal change in the anterior lobe of the dwarf mouse

occurs at some time or other between the 20th fetal day and the 12th day after birth, manifesting itself by an increase in the numbers of small pyknotic acidophiles and small pyknotic chromophobes, and a corresponding reduction in typical acidophiles. This stage is probably preceded by one in which there is seen only a slight increase in the number of small pyknotic cells.

The growth and formation of the pituitary has a uniform and parallel course in normals and dwarfs until 5 days after birth. From the 2th day, the anterior lobe is markedly hypoplastic in the dwarfs, whose anterior lobe is subject to the most pronounced parenchymal changes.

A hypothesis is set up as to the cell cycle in the anterior lobe of the normal mouse and the dwarf mouse. Instead of being transformed into hypoacidophiles, typical acidophiles, large acidophiles and hyperacidophiles, the typical chromophobe cells of the dwarf are transformed direct into hyperacidophiles and small pyknotic acidophiles and further from that stage into small pyknotic acidophiles and further from that stage into small pyknotic chromophobes («a proportional change to the right»). The nucleus of the glandular cell in the anterior pituitary is getting pyknotic *i. e.* it begins to degenerate at the same moment at the cytoplasm of the cell begins to become acidophile, that is the cell begins to work as a hormone-producing glandular cell.

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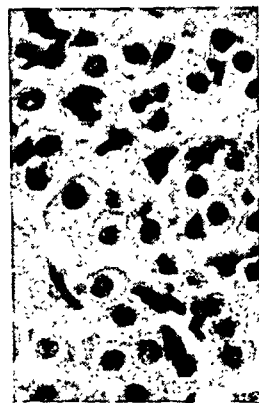
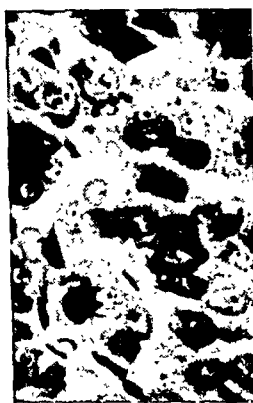
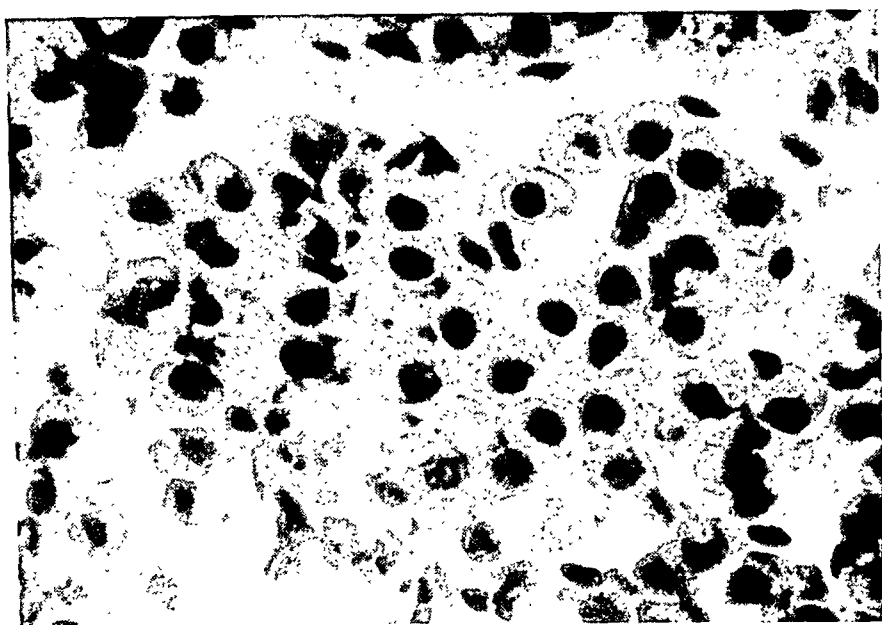
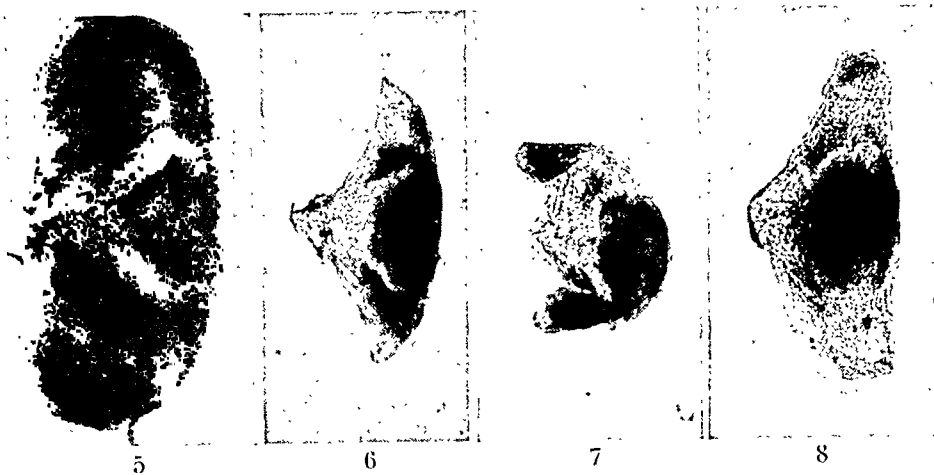


Fig. 5-8. Isolated pituitaries from a normal mouse and from 3 dwarfs.
Scale see fig. 22.

Fig. 9. Anterior lobe of normal mouse. 1400 \times . Hematoxylin-eosin.

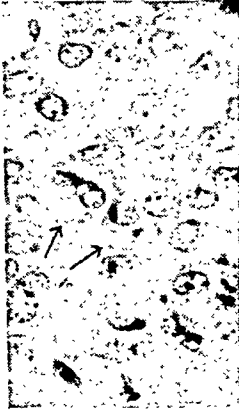
Fig. 10. Typical acidophiles. Normal mouse. 900 \times . Hematoxylin-eosin.

Fig. 11. Typical chromophobes and typical acidophiles. Normal mouse. 900 \times . Iron-hematoxylin.

Fig. 12. Typical acidophiles and large acidophiles. Normal mouse. 900 \times . Hematoxylin-eosin.



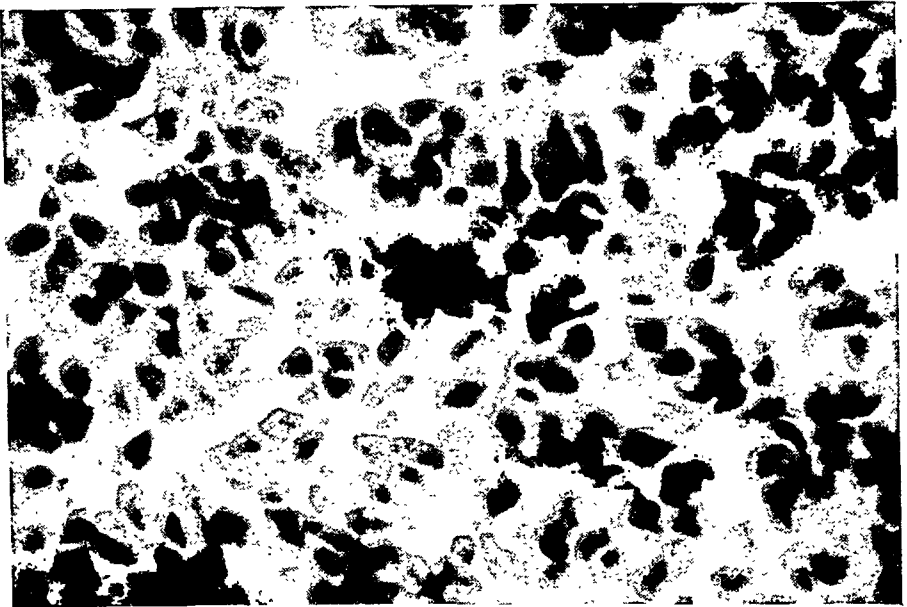
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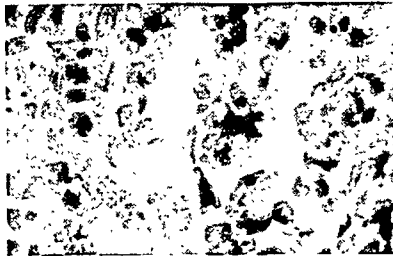
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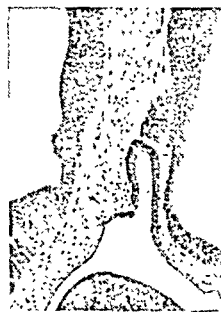


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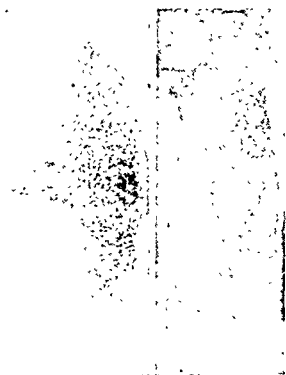
- Fig. 13.* Basophiles. Normal mouse. 900 \times . Hematoxylin-eosin.
Fig. 14. Pregnancy cells. Normal mouse. 900 \times . Hematoxylin-eosin.
Fig. 15. Colloid cyst. Normal mouse. 900 \times . Hematoxylin-eosin.
Fig. 16. Anterior lobe of dwarf mouse. 1400 \times . Hematoxylin-eosin.
Fig. 17. Small pyknotic acidophiles. Dwarf mouse. 900 \times . Hematoxylin-eosin.



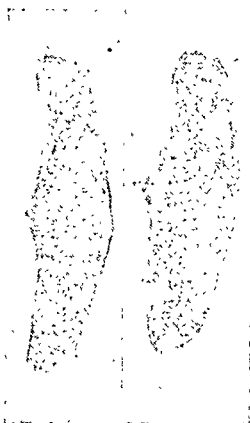
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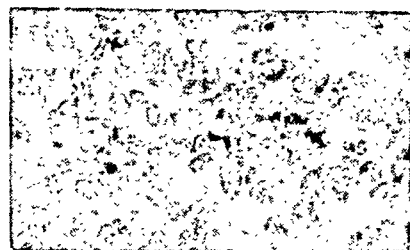
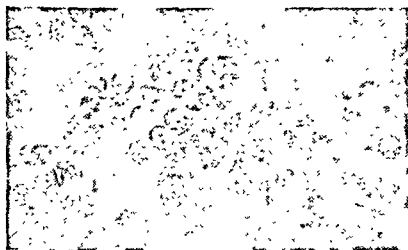
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Fig. 18 & 19. Pituitary, 11th fetal day. Sagittal section, 65 \times . Hematoxylin-eosin.

Fig. 20. Isolated pituitaries, 5 days after birth. Left from normal mouse. Right from dwarf.

Fig. 21. Isolated pituitaries, 6 days after birth. Left from dwarf. Right from normal mouse.

Fig. 22. 1 mm.

Fig. 23. Anterior lobe parenchyma from normal mouse (left) and dwarf (right), 9 days after birth. 900 \times . Hematoxylin-eosin.

STUDIES ON HEREDITARY DWARFISM IN MICE. VII*)

EXPERIMENTALLY PRODUCED CHROMOPHOBE ANTERIOR PITUITARY LOBE ADENOMAS IN DWARF MICE TREATED WITH SEX HORMONES INVESTIGATIONS INTO THE MECHANISM PRODUCING THE CHROMOPHOBE ANTERIOR LOBE ADENOMA

By *Torben Francis*, M.D.

(Received for publication October 23rd 1944).

Chromophobe anterior lobe adenomas can be brought about experimentally in animals by prolonged, continual ingestion of estrin. This observation was made in 1936 in three different places independent of each other, viz. by *Cramer & Horning* in England, by *Zondek in Palestine*, and by *Mac Euen, Selye & Collip* in the U. S. A., and numerous writers have since borne out this observation. The estrin treatment lasts on an average 10 or 11 months, generally not under 7 months, and in the treated mice, rats, and chickens it soon leads to growth retardation of a typical anterior pituitary character as well as to hypoplasia and atrophy of the sexual glands, and after about 10 months of treatment there appear large, greatly vascularized anterior pituitary adenomas of a typical chromophobe character.

On this basis we should naturally presume that estrin treatment should be able to affect the defective anterior lobe parenchyma of the dwarf mouse and bring about changes here that, compared with the changes in the pituitaries of the normal estrin-treated animals, might elucidate the question as to the mechanism producing the chromophobe anterior lobe adenoma, and, besides, give interesting information on the response of the abnormal and incomplete cell cycle of the dwarf mouse to treatment with estrin.

*) Previous publications in this series: *Acta path. et microbiol. Scand.* XIII, 512, 36 — XIV, 197, 37 — Suppl. XXXVII, 290, 38 — XVIII, 20, 41 — XVIII, 169, 41 — XIX, 563, 42 — XXI, 928, 44.

For further particulars see the author's monograph: *Investigations into the development of the pituitary at hereditary anterior pituitary dwarfism in mice with reference to the pathogenesis of the anterior pituitary dwarfism.* pp. 154. Munksgaard. Copenhagen. 1944.

To Miss P. A. Brandt's Legacy for the Promotion of Pathologic-physiological Research and Physiologic-gymnastic Science and to The Danish National Anti-Cancer League. I am indebted for financial support.

Technique.

The estrin preparations applied are the synthetic, estrogenic substances Hexoestrol and Östroxan (Alfred Benzon). Besides some dwarfs have been treated with testis hormone in the form of testosterone propionate (Testoviron Schering) in order to make out whether the male sex hormone has the same influence on the cytology of the anterior pituitary lobe of the dwarf mouse as the female sex hormone.

Fixation: Helly's fluid. 10 per cent formol.

Staining: Hematoxylin-eosin.

Material. 6 normal female mice and 2 normal male mice between the ages of 40 and 87 days have been treated for periods of from 21 to 71 days with hexoestrol or östroxan in total doses of from 18 to 60 mgm, 2 mgm having generally been injected subcutaneously 3 times weekly (table 1).

The treated dwarf mice comprise 12 males and 3 females. 7 of these dwarfs have been treated with hexoestrol or östroxan in doses of from 8 to 92 mgm for periods ranging from 8 to 119 days, i. e. treatment with a smaller or larger dosis for periods corresponding to the periods of treatment of the normal animals. 6 dwarfs have been treated with hexoestrol or testosterone propionate in doses of from 10 to 20 mgm for very short periods. Thus 2 of them were killed already 2 hours after the first injection, 1 was killed 26 hours after the first injection, and 3, who had within 26 hours been treated by 2 injections of 10 mgm testosterone propionate each, were killed 2 hours after the latter injection (table 2). Finally 2 dwarfs have been treated with hexoestrol and östroxan, the total dosis being 10 and 52 mgm respectively. The dwarf that had got only 10 mgm was killed on the 3rd day after the treatment, while the dwarf that had received 52 mgm, over a period of 60 days, was killed 5 days after the last injection (table 3).

The epithelium in the anterior lobe of the normal mouse and the dwarf mouse is described by the author in previous papers (1944), in which also a hypothesis as to the cell cycle in the anterior lobe of the normal mouse and the dwarf mouse is set up. In the normal mouse the two main groups of cells are the chromophobes and the acidophiles, whereas basophiles occur but rarely.

The chromophobes occur in the following forms: Typical chromophobe cells, which constitute about 40 % of the anterior lobe cells, and small chromophobe cells with a pyknotic nucleus (small pyknotic chromophobe cells), which constitute never more than 3 to 6 per cent of the anterior lobe cells.

The acidophiles are found in the following forms: Hypoacidophile cells, typical acidophile cells, large acidophile cells, hyperacidophile cells and small acidophile cells with a pyknotic nucleus (small pyknotic acidophile cells). The acidophiles constitute 45 to 55 per cent of the anterior lobe cells the typical acidophile cells being the predominant element.

A schematic presentation of the author's hypothesis as to the cycle in the anterior lobe of the normal mouse is given in fig. 1: The typical chromophobe cell or mother cell (A) is first transformed into a hypoacidophile (B), which is the first cell to occur in the anterior lobe of the fetus after the formation of the typical chromophobes. From the hypoacidophile the typical acidophile (C) is developed, the

latter being not found till after the presence of the former has been demonstrated (the 15th fetal day).

The typical acidophile may a) fill with secretion and develop into a large acidophile (D) or b) liberate its secretion and develop into a hyperacidophile (E); possibly it may also c) degenerate direct into a small pyknotic acidophile (F); yet another possibility is d) that it is transformed into a basophile (G).

The large acidophile (D) may by liberation of some of its secretion redevelop into a typical acidophile (C), or by liberation of the greater part of its secretion be transformed into a hyperacidophile (E).

The hyperacidophile (E) may by a renewed hormone production redevelop into a typical acidophile (C) and thus again take part in the cycle, or it may by further draining off be transformed into a small pyknotic acidophile (F).

The small pyknotic acidophile (F) is an acidophile cell with a minimum of function. In by far the greater number of cases it is transformed into a small pyknotic chromophobe (H), which is the final stage in the cycle. But it may possibly in a few cases redevelop into a typical acidophile and thus again take part in the cycle.

The basophile (G) does not take a regular part in the cycle. Probably it is developed from the typical acidophile (C) like the hyperacidophile, which it resembles a great deal both in structure and size, and presumably it may redevelop into a typical acidophile.

Fig. 2 shows the author's hypothesis as to the cycle in the anterior lobe of the dwarf: The anterior lobe consists exclusively of some few typical chromophobes, a somewhat varying number of hyperacidophiles, and numerous small pyknotic acidophiles and chromophobes, and the cycle in the anterior lobe of the dwarf mouse presents »a proportional change to the right«: All the typical chromophobes (A), the number of which is greatly reduced, are most frequently transformed direct, but also occasionally by the hyperacidophile stage (E) into small pyknotic acidophiles (F) and small pyknotic chromophobes (H).

This means, that the nucleus of the glandular cell in the anterior pituitary of the dwarf mouse is getting pyknotic i. e. begins to degenerate in the same moment in which the cytoplasm of the cell begins to become acidophile i. e. the cell begins to work as a hormone producing cell.

Observations. In the normal, estrin-treated mice the following may be observed (table 1):

- 1) Complete cessation of growth. A variation of weight of ± 0.5 gm.
- 2) Hyperemia of pituitary, the other endocrine glands, liver, and spleen.
- 3) A slight increase in the number of typical chromophobes in the anterior pituitary lobe of the two mice treated with the largest

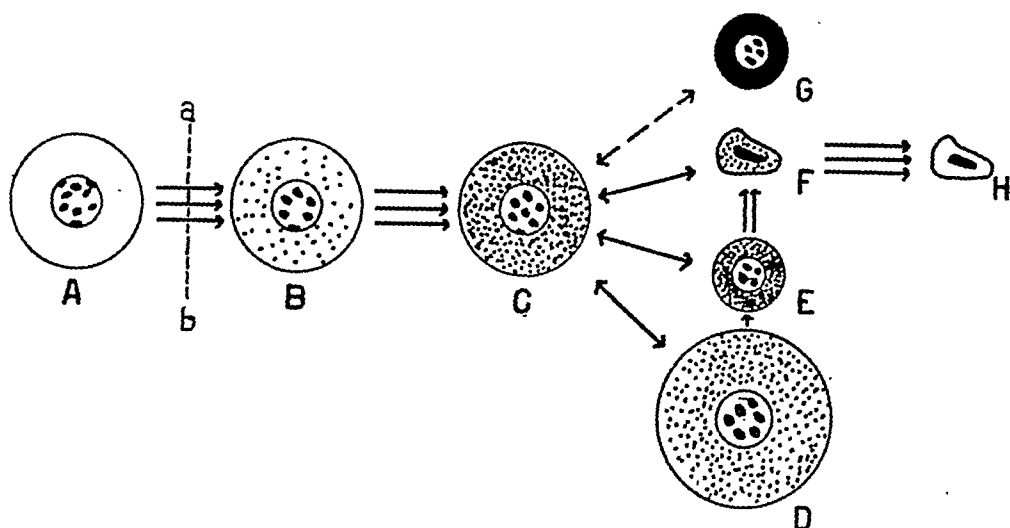


Fig. 1.

Cycle in the anterior lobe of normal mice.

A typical chromophobe

B hypoacidophile

C typical acidophile

D large acidophile

E hyperacidophile

F small pyknotic acidophile

G basophile

H small pyknotic chromophobe

a ———— b checking of the cycle by sex hormones.

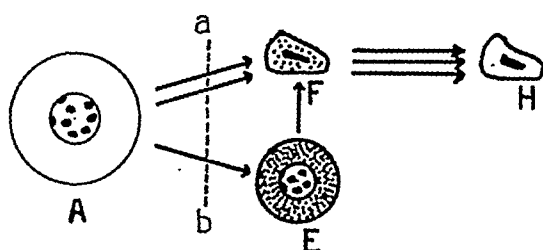


Fig. 2.

Cycle in the anterior lobe of dwarf mice.

A typical chromophobe

E hyperacidophile

F small pyknotic acidophile

H small pyknotic chromophobe

a ———— b checking of the cycle by sex hormones.

doses for the longest periods (364 and 382). Most of the nuclei of the typical chromophobes are large and poor in chromatin.

In the 13 dwarfs killed while under treatment with sexual hormone the following may be observed (table 2):

- 1) The already minimal growth is brought completely to a standstill. The weight loss may amount to 2.5 gm (385).
- 2) Hyperemia of pituitary, the other endocrine glands, liver, and spleen. The vessels of the anterior and the posterior lobe of the pituitary are dilated and contain large amounts of acido-

Table 4.

The glandular cells of the anterior pituitary lobe of normal mice at treatment with estrogenic or androgenic hormones.

No.	347	349	358	377	384	361	364	382
Age (Days)	40	53	67	77	80	87	87	87
Sex (δ = male, \varnothing = female)	\varnothing	\varnothing	\varnothing	\varnothing	\varnothing	\varnothing	\varnothing	\varnothing
Total dosis (mgm)	18	33	45	48	48	48	48	48
Lenght of treatment period (D = Days, H = Hours)	21 D	39 D	52 D	60 D	60 D	56 D	56 D	70 D
Hormone preparation (H = Hexoestrol, \varnothing = Østrozan, T = Testoviron)	\varnothing	H	H	\varnothing	\varnothing	H	H	\varnothing
Weight increase (+) or weight loss (—) during the experimenting period in gm.	0	+ 0,5	— 0,5	+ 0,5	0	— 0,5	— 0,5	+ 0,5
ANTERIOR LOBE CELLS								
Typical chromophobes	++	++	++	++	++	++	++	++
Hypoadidophiles	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)
Typical acidophiles	+++	+++	+++	+++	+++	+++	+++	+++
Large acidophiles	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)
Hyperacidophiles	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)
Small pyknotic acidophiles	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)
Small pyknotic chromophobes	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)
Basophiles	\varnothing	\varnothing	\varnothing	\varnothing	\varnothing	\varnothing	\varnothing	\varnothing

Table 3.

The glandular cells of the anterior pituitary lobe of mice with hereditary anterior pituitary dwarfism at treatment with estrogenic or androgenic hormones.

No.		604	388
Age (Days)	Un- treated dwarf mouse	37	63
Sex (♂ = male, ♀ = female)		♂	♂
Total dosis (mgm)		10	52
Treatment period (H = Hours, D = Days)		1 D	60 D
Number of days (D) without treatment after expiration of treatment period		2 D	5 D
Hormone preparation (H = Hexoestrol, Ø = Østrofan)		H	Ø
Weight increase (+) or weight loss (—) during the experimenting period in gm.			○

ANTERIOR LOBE CELLS

Typical chromophobes	(+)	(+)	(+)
Hypoacidophiles	○	○	○
Large acidophiles	○	○	○
Typical acidophiles	○	○	○
Hyperacidophiles	(+)	(+)	(+)
Small pyknotic acidophiles	+++++	+++++	+++++
Small pyknotic chromophobes	+++++	+++++	+++++
Basophiles	○	○	○

philic colloid, which in the form of bands fill up the vessels. In the colloid the red corpuscles lie arranged in rouleaux.

3) The glandular parenchyma of the anterior pituitary lobe undergoes a definite change (fig. 3 and table 2):

- The hyperacidophiles disappear completely already after 2 hours of treatment.
- The numbers of small pyknotic acidophiles and small pyknotic chromophobes decrease considerably already after 2 hours of treatment. After 26 hours of treatment only a few are found left. After a still longer period of treatment — 56 days or more — they disappear practically completely.
- The number of typical chromophobes increases very considerably already after 2 hours of treatment. After 26 hours of treatment the typical chromophobes predominate the cell

picture, and the structure of the anterior pituitary lobe of the dwarf mouse is now exactly that of the typical chromophobe adenoma. After a still longer period of treatment — 56 days or more — the anterior lobe of the treated dwarf mouse consists exclusively of typical chromophobes most of them with a large nucleus containing a medium amount of chromatin while some of the nuclei are smaller with a compact structure.

This change in the anterior pituitary lobe of the dwarf mouse is equally pronounced all over the lobe. Its shape and size is unaltered, and there is seen no change in the number of mitoses.

- 4) a. Thyroid. The thyroid of the dwarf mouse is previously reduced in size and has a very flat follicular epithelium. The follicular epithelium in the thyroids of the treated dwarfs is also very low, but besides there is often seen colloid stasis (348, 363). Follicles filled with colloid are also seen in some of the normal, treated mice.
 - b. Parathyroids. The cellular structure is not changed. There is some hyperemia.
 - c. Thymus. Hyperemia.
 - d. Adrenals. The adrenal cortex shows no changes besides the hyperemia and stasis. The zone X and the medulla are also unaltered.
 - e. Testes. The number of mitoses in the seminal vesicles seems to be considerable, and besides many giant cells are seen (362, 365, 370, 385, 386, 388, 332). The interstitial tissue is unaltered. There is hyperemia and stasis.
- Ovaries. In all the ovaries of the treated dwarfs is seen hyperemia and in a single ovary is seen atrophy.
- f. Liver and spleen. Hyperemia and stasis. No changes in the parenchyma.

In the 2 dwarfs not killed till after the discontinuation of the treatment (table 3) the following may be observed:

- 1) Slight hyperemia in the anterior pituitary lobe.
- 2) The anterior lobe parenchyma consists of numerous small pyknotic acidophiles and numerous small pyknotic chromophobes, which predominate the picture. Besides there are found a few typical chromophobes and a few hyperacidophiles, i. e. exactly the same picture as in the untreated dwarf mouse.

Conclusions. The influence of the sex hormones on the anterior pituitary lobe must then be supposed to be as follows, the cell changes in the anterior lobe of the treated dwarf mouse serving as a clue to the understanding of the mode of action of the hormones:

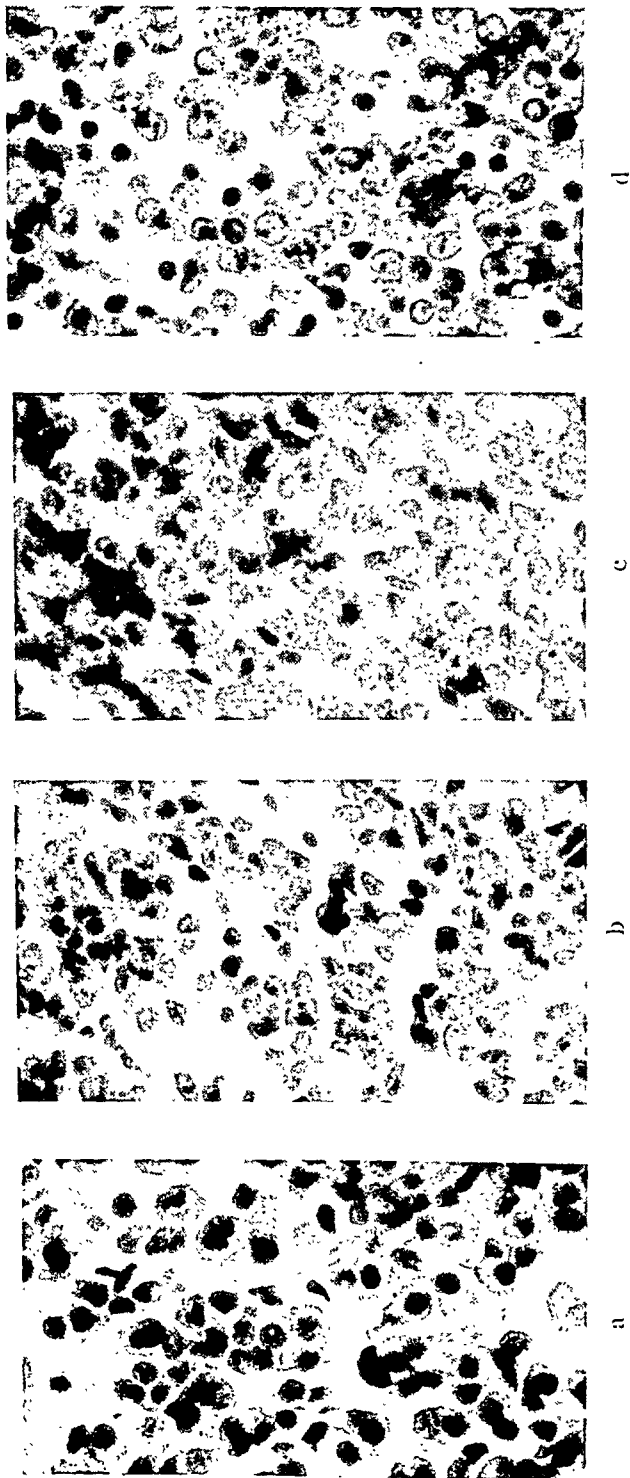


Fig. 3.
Anterior pituitary lobe of dwarf mouse treated with sex hormone.

- a. Normal, untreated mouse (No. 240).
- b. Untreated dwarf mouse (No. 275).
- c. 12 days old female dwarf mouse treated for 26 hours with 20 mgm testosterone propionate (No. 607, table 2).
- d. 87 days old female dwarf mouse treated for 56 days with 48 mgm Hexoestrol (No. 363, table 2).

Hematoxylin-eosin. 1100 X.

- 1) The sex hormones have a disturbing influence on the cell cycle of the anterior pituitary lobe.
- 2) The disturbance itself consists in a checking of the cycle, which prevents the typical chromophobes from entering into the cycle (fig. 1, 2).
- 3) Instead of »a proportional change to the right« the dwarf mouse treated with sex hormones gets »a proportional change to the left«.

Instead of a normal cell picture the normal mouse treated with sex hormones gets »a proportional change to the left« too.

- 4) The sex hormones prevent in the dwarf the »proportional change to the right« i. e. prevent the abnormally quick degeneration of the nuclei of the cells; besides the sex hormones prevent the cytoplasm of the cells in getting acidophile i. e. becoming hormone-producing glandular cells.
- 5) In the treated dwarf mouse the checking of the cycle is observable already after a few hours of sex hormone influence, and already after a short period of treatment the anterior lobe of the dwarf mouse proves to be transformed into a chromophobe adenoma. Thus the defective cycle in the anterior lobe of the dwarf mouse with »a proportional change to the right« is extremely affectible to hormonal disturbances, unlike the normal cycle in the anterior lobe of the normal mouse, which seems to be far more stable.
- 6) The cellular change seems to be reversible, as the original picture is found in the dwarfs whose anterior lobe is not examined till a point of time at which the influence of the sex hormones must be supposed to have ceased.

Thus presumably the forming and growth of the chromophobe pituitary adenoma brought about experimentally takes place in the following manner:

- 1) The forming of the adenoma is due to the checking of the cycle, by which the anterior lobe tissue is prevented from producing hypoacidophiles, typical acidophiles, large acidophiles, hyperacidophiles, small pyknotic acidophiles, and small pyknotic chromophobes.
- 2) The growth of the adenoma is due to the fact that the normal cell excretion from the chromophobe cell element, the mass of which increases on account of a normal mitotic cell division, is prevented, i. e. the growth of the adenoma is due to a normal tissue proliferation + prevention of the normal cell excretion.

Discussion. We cannot justly from these investigations into the mechanism of producing experimentally the chromophobe anterior lobe adenoma in mice conclude very much as to the pathogenesis of

the human, spontaneously developed chromophobe pituitary adenoma, especially it is not permissible to parallelize the tumour formations of humans and those of mice, these tumours being in many respects widely different.

On the other hand the cytological pictures of the two above-mentioned chromophobe adenomas resemble each other so much (the most important difference is the pronounced hyperemia in the adenoma brought about experimentally, and this hyperemia is no doubt chiefly due to a general stasis in the organism of the hormone-treated animal) that it must be justifiable on the basis of the mechanism producing the adenoma brought about experimentally to set up a hypothesis as to the pathogenesis of the spontaneous, human adenoma.

At the forming of the adenoma brought about experimentally two factors seem to be of vital importance. One is the hormonal, cycle-disturbing factor (estrogenic or androgenic hormone ingested in excess) checking the cycle. The other is the condition of the anterior lobe tissue, i. e. the composition and cell cycle of the glandular tissue. If the composition of the glandular tissue is normal with a normal cell cycle large doses of estrogenic or androgenic hormone are required for a great length of time to check the cycle so much that a chromophobe adenoma is produced. But if, as is the case with the dwarf mouse, the cycle is defective and incomplete, only a few hours of ingestion of estrogenic or androgenic hormone are necessary to bring about a very marked checking of the cycle, which after a short period of treatment transforms the anterior lobe into a chromophobe adenoma.

It is rather unlikely that the human organism under normal or pathological conditions should be able to produce estrogenic or androgenic hormone in such amounts as to check the normal, probably very stable cell cycle in the anterior pituitary lobe so much that a chromophobe adenoma could be produced. But if we imagine that somewhere in the anterior pituitary lobe of a human being there is in advance a local cycle disturbance, for instance in the form of »a proportional change to the right« — as in the dwarf mouse — it is very well thinkable that a hormonal disturbing factor (in the form of hormones from inferior endocrine glands) may get a checking influence on the unstable cycle and by »a proportional change to the left« lead to the forming of a chromophobe adenoma in this place.

The following particulars seem to be in favour of the fact that a cycle disturbance with »a proportional change to the right« and an unstable cycle, as in the dwarf mouse, may occur under certain circumstances in the normal anterior pituitary lobe of the normal organism:

1. As shown by *Collin & Hennequin* (1936) extirpation of the superior cervical ganglion, from where the sympathetic nerves together with the vessels are led to the pituitary, brings about that the chromo-

phile cells disappear temporarily from the anterior pituitary lobe, which then gets to consist chiefly of small glandular cells with a pyknotic nucleus, i. e. a parenchyma change that according to the description resembles very much the proportional change to the right« of the dwarf mouse.

2. *Barrie* (1937) has in young rats whose mothers are kept on a diet poor in vitamin E found degranulation in the anterior lobe of the chromophiles, which are small with pyknotic nuclei.

3. According to *Westman* (1943) transection of the pituitary stalk in rats brings about complete absence of acidophile cells in the anterior pituitary lobe, the cells of which become poor in protoplasm and homogeneous. In illustration of this observation *Westman* has presented a picture of the anterior lobe parenchyma of a rat with transected pituitary stalk (*Nord. Med.* 20, 1784, fig. 5, 1943). This resembles so much the anterior pituitary lobe of the dwarf mouse with its numerous small pyknotic cells that it must be regarded as very likely that a breaking off of the connection between the hypothalamus and the pituitary — and on the whole disturbances of the function of the hypothalamus — may lead to »a proportional change to the right« in the anterior pituitary lobe of an otherwise normal organism.

4. As mentioned in the author's monograph (1944) the anterior lobe of the human anterior pituitary dwarf with »simple anterior lobe hypoplasia« contains numerous small glandular cells with dark, angular nuclei rich in chromatin; and in dwarfs with anterior pituitary dwarfism on account of anterior lobe hypoplasia with a tumour formation the anterior lobe rest consists in by far the greater number of cases of small pyknotic glandular cells, which have generally been regarded as expressive of pressure atrophy, but which in the author's opinion is rather an expression of a proportional change in the cell numbers with »a proportional change to the right«.

5. The spontaneous, chromophobe, anterior lobe adenomas in humans often contain numerous small pyknotic cells.

Summary.

By treatment of the hereditary anterior pituitary dwarf mouse with synthetic sex hormones (Hexoestrol, Østroxan and Testoviron) the anterior pituitary lobe is transformed decisively in the course of a few hours. The number of typical chromophobes increases considerably, that of small pyknotic acidophiles and small pyknotic chromophobes decreases greatly, and the anterior lobe of the dwarf mouse is transformed into a chromophobe adenoma. The sex hormones seem to have a checking influence on the cycle in the anterior pituitary lobe, and this checking is far more pronounced in the anterior pitui-

tary dwarf mouse than in the normal mouse. 2 factors must be supposed to play a part in the pathogenesis of the chromophobe anterior lobe adenoma. One is a hormonal disturbing factor, the other is the stability of the anterior lobe cycle to hormonal disturbances.

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RECHERCHES SUR LA FERMENTATION MUTATIVE DES BACTÉRIES. (5^e communication).

Par *Martin Kristensen*.

(Reçu par la rédaction le 30 octobre 1944).

XIII. FERMENTATIONS MUTATIVES CHEZ DES BACTÉRIES COLIFORMES

Au cours des années, beaucoup d'auteurs ont rapporté la découverte de bâtonnets ne prenant pas le Gram qui fermentent lentement le lactose ou le saccharose, en beaucoup de cas d'une façon mutative. A cause de l'importance spéciale que présente le lactose pour le diagnostic pratique de colibacilles et de bactéries intestinales pathogènes, c'est tout particulièrement ce sucre qui fait l'objet d'attention.

Parmi les recherches faites pendant les dernières années nous mentionnons celles de *Kennedy, Cummings & Morrow*, de *Dulaney & Michelson* et de *Lewis & Hitchener*; chacun de ces trois groupes d'auteurs rapporte la découverte fréquente de bâtonnets qui fermentent le lactose d'une façon mutative, respectivement dans les matières fécales et les urines humaines, dans les matières fécales à la diarrhée chez nourissons et dans les matières fécales de poulets. La présence tant de souches mutantes que de souches à fermentation lente primaire dans le même matériau a été rapportée par *Sandiford* (matières fécales humaines) et *Stuart, Mickle & Borman* (lait, terre, blé et matières fécales), tandis que *Jones, Orcutt & Little* n'ont trouvé, dans les matières fécales de vaches, que des souches à fermentation lente primaire. *Deere, Dulaney & Michelson* ont montré que la forme originaire qui, pratiquement parlant, ne provoque pas de fermentation à un essai de culture ordinaire dans un milieu à lactose (examiné par le dosage direct de la teneur en lactose du milieu nutritif) contient pourtant de la lactase, bien que dans une quantité plus faible que la forme mutée. On peut constater l'existence de la lactase, même dans les cultures qui n'ont pas été en contact avec le lactose, mais on la trouve dans des quantités beaucoup plus grandes dans les cultures d'un milieu à lactose, voire en plus grande quantité que dans les cultures de la forme mutée, si celles-ci n'ont pas été cultivées directement d'un milieu à lactose. Dans des conditions d'essai égales, la forme mutée contenait pourtant plus de lactase que la forme originaire. L'expérience de *Mikkelsen* avec les souches à lente fermentation de lactose est étrange; il est possible que l'emploi du milieu nutritif d'*Endo* joue un rôle. Il paraît qu'on peut résumer ses constations comme suit: Les souches possédaient, au stade primaire, un certain pouvoir fermentatif et en outre elles étaient capables de muter. Le mutant ne devenait pourtant pas un ferment typique

mais était seulement capable de muter un peu plus vite que la culture originaire.

Il faut se rappeler que si principalement on n'emploie l'acidification que comme signe de fermentation — ainsi que c'est le cas pour nos propres recherches — on peut risquer que beaucoup de fermentations faibles passent inaperçues. Lorsque nous parlons ci-après de cultures qui ne fermentent pas un sucre donné, cela veut seulement dire qu'il n'est pas question d'une fermentation avec une acidification distincte. Par contre nous sommes d'avis que l'acidification dans un milieu contenant du lactose (ou du saccharose) avec la technique employée ici est un signe certain de fermentation.

Dans l'ouvrage de *Kristensen, Bojlén & Kjær* on a examiné 1004 souches de bactéries coliformes entre autre choses envers le lactose et le saccharose; on a constaté chez 711 souches une fermentation tardive de lactose et chez 297 souches une fermentation tardive de saccharose. Cependant nous n'avons pas examiné, si cette fermentation était de caractère typique ou mutatif. Afin d'en obtenir une idée plus précise, on a choisi les souches, qu'on avait gardées, de 14 »types« qui à l'examen originel avaient donné une fermentation tardive tant de lactose que de saccharose. On a entrepris une dissémination sur une plaque à lactose et à saccharose ainsi qu'un ensemencement dans un liquide nutritif à lactose et à saccharose; en cas de fermentation dans un des verres on a fait, de celui-ci, une dissémination sur une plaque à la même espèce de sucre que contenait le verre. Pour contrôler si la mutation présumée était authentique et non pas due au mélange de deux bactéries d'origine différente, on a ensemencé, de chaque souche qui donnait tant des colonies bleues que des colonies jaunes, au moins une colonie de chaque sorte dans toute une série de milieux nutritifs (adonite, dulcite, sorbite, xylose, rhamnose, maltose, salicine, »bouillon-indol«) envers lesquels des différences entre les bacilles coliformes (ou les colibacilles) entre elles se font valoir. Chez une des souches (type 45) on a constaté des diversités considérables entre les différentes formes; ces différences pouvaient être retrouvées à l'ensemencement de la culture de bouillon qui n'avait pas encore été ensemencée dans le lactose ou le saccharose. Il s'agissait probablement d'un mélange de deux souches de bactéries toutes différentes. En conséquence, nous ne nous occuperons plus de cette »souche«. Ci-après nous désignerons les souches par les numéros qui sont, en réalité, les numéros des types. En ce qui concerne les autres 13 souches, la colonie bleue et la colonie jaune donnaient toujours des réactions concordantes qui, il est vrai, ne correspondaient pas toujours aux types de fermentation qu'indiquaient les numéros des types. Quelques-uns des mutants en lactose ont été ensemencés en saccharose et inversement; de même que c'était le cas pour les autres sucres, il s'est aussi montré ici que la mutation envers une espèce de sucre n'entraîne pas de différence dans la réaction envers les autres

sucres. Comme mentionné antérieurement et ci-dessous, nous avons pourtant vu des exceptions à cette règle. En outre on a ensemencé en mannite avec un verre intérieur. Dans cette espèce de sucre, une acidification s'est produite dans tous les cas au cours de 24 heures. En ce qui concerne la formation d'air, les colonies bleues et jaunes des souches 18, 24, 52, 60, 64, 82 et 109 ont produit à peu près la même quantité d'air; ni les colonies bleues ni les colonies jaunes des souches 36, 76 et 80 n'ont produit de l'air; le mutant (en lactose) de 96 a produit une quantité d'air considérablement plus grande que la forme non mutée; le mutant (en saccharose) de 79 a produit, au cours de 48 heures, $\frac{1}{20}$ de volume d'air tandis que la forme non mutée n'a pas produit d'air.

En laissant à part les détails techniques spéciaux, nous donnons ci-après un exposé sommaire du résultat de l'examen de chaque souche:

1. *Réaction envers lactose.*

18. Fermentait tardivement mais mutait dans une forme à fermentation rapide qui ne s'est pas manifestée, cependant, que par des traits jaunes là où l'ensemencement de la culture avait été spécialement dense et qui ne formait évidemment qu'une partie très minime de la quantité totale de la culture. On a procédé à une nouvelle dissémination sur une plaque à lactose de la partie de la culture à la couleur jaune la plus pure, et cela a été répété dans deux nouvelles générations, mais ce n'était toujours qu'une très petite partie de la culture qui prit la couleur jaune. Il s'agit donc d'une mutation à une très grande réversibilité.

24 et 76 fermentaient tardivement mais mutaient vite dans une forme à fermentation rapide qu'on pouvait isoler à l'état de pureté; cela est aussi le cas pour les mutants mentionnés ci-après à moins d'observations contraires.

36. La forme primaire ne fermentait pas (par là nous entendons ici et ci-après que les colonies sur la plaque à lactose — abstraction faite de formation de bourgeons — ont gardé la couleur bleue à l'observation pendant 5—7 jours) mais mutait vite dans une forme à fermentation rapide.

52. Il y avait deux différents degrés de colonies à fermentation tardive. A la dissémination d'un liquide nutritif, des colonies à fermentation rapide se sont produites.

60. Il y avait un mélange de colonies qui ne fermentaient pas et de colonies qui fermentaient rapidement. Celles à fermentation rapide donnaient après dissémination, de façon prépondérante, des colonies bleues, seulement un petit nombre de colonies jaunes; à la dissémination continuée on pouvait pourtant obtenir les colonies jaunes à l'état de pureté.

64, 82 et 104 donnaient exclusivement des colonies à fermentation assez lente.

79 contenait deux différents degrés de colonies qui fermentaient lentement.

80 se comportait à l'examen macroscopique ordinaire comme 79, mais en l'examinant de plus près à la loupe on constata que la coloration en jaune tardive était due à une mutation précoce avec de nombreux colonies secondaires dans chaque colonie.

96 donnait, à la dissémination sur plaque, un mélange de colonies à fermentation rapide et lente.

109 ne fermentait pas du tout.

2. Réaction envers saccharose.

18, 64, 82 et 109 ne montraient pas de fermentation primaire mais mutaient dans une forme à fermentation rapide.

24 donnait à la dissémination un mélange de colonies à fermentation rapide et lente.

36, 60, 96 et 104 ne fermentaient pas du tout.

52 poussait avec des colonies bleues seulement. Dans la partie serrée de la végétation, une colonie jaune solitaire apparut le 7^e jour; à la dissémination sur une nouvelle plaque à saccharose, celle-là fermentait en 24 heures. A cause de l'apparition de la forme fermentaire dans la végétation dense on ne pouvait pas décider s'il s'agissait d'un individu fermentaire présent depuis l'origine ou d'un mutant; la dernière présomption est pourtant la plus probable en considération du fait que cette colonie se manifesta si tardivement bien qu'à l'état de pureté elle fermentât rapidement.

79 ne montrait pas de fermentation primaire mais mutait soit dans une forme à fermentation rapide, soit dans une forme à fermentation lente.

76 et 80 se comportaient envers le saccharose de la même façon que 80 envers lactose.

De l'examen de la réaction de ces 13 souches envers le lactose et le saccharose il résulte clairement qu'on ne peut pas grouper ces fermentations tardives dans une formule commune. Tantôt il s'agit d'une fermentation lente primaire, tantôt d'une fermentation mutative et cette dernière peut, de son côté, se produire tantôt sur la base d'une forme non fermentaire, tantôt sur la base d'une forme à fermentation lente.

Un fait spécialement remarquable est l'apparition d'un mélange de la forme fermentaire et de la forme non fermentaire chez les souches 60 et 24 avant l'entrée en contact de celles-ci avec le lactose, respectivement le saccharose. Nous ne pouvons pas décider, il est vrai, s'il s'agit d'une mutation positive chez une forme autrement non fermentaire (cfr. vol. 17 p. 197—198 (*Henderson Smith, Lewis*) et 226) ou d'une mutation déficitaire chez une souche fermentaire.

Afin d'observer, si possible, l'apparition ou de la forme «positive» ou bien de la forme «négative», il y a été produit de la souche 24 une culture unicellulaire selon Ørskov tant de la forme non fermentant le saccharose que de la forme fermentant le saccharose. On a procédé à la dissémination sur une plaque à saccharose immédiatement et après 3 et 5 mois. Dans tous les cas, la première forme ne donnait que des colonies bleues, la dernière forme seulement des colonies jaunes. Cela ne contredit pas l'authenticité de la dissociation, puisque celle-ci peut très bien s'être produite au cours des env. 10 ans pendant lesquels les cultures ont été maintenues, bien qu'elle ne se manifeste pas dans le courant de quelques mois dans une culture unicellulaire quelconque. La question de savoir s'il ne s'agit pas d'un mélange fortuit de deux différentes souches résulte — comme déjà mentionné — du fait que les deux formes se sont comportées de tout à fait la même façon à leurs autres réactions biochimiques. Tant la culture originaire, qu'une culture de la colonie bleue et une culture de la colonie jaune ont fermenté la mannite, la dulcité, la sorbite, le xylose et le rhamnose au cours de 24 heures; dans le maltose nous avons constaté une vague coloration en jaune le 1^{er} jour, une forte coloration en jaune le 2^e jour. Aucune des cultures des autres 12 souches ne s'est comportée de cette manière.

Les expériences quant à l'«entraînement» mentionnées dans la communication précédente (vol. 21, p. 232) ont été réitérés avec une

partie des cultures coliformes; on a examiné les fermentations qui étaient lentes, faibles ou qui, par opposition à l'examen primitif, avaient donné un résultat négatif.

Dans une première série, on a ensemencé des verres avec les sucres en question tant d'une culture de bouillon (ensemencée directement de piqûre) que d'une culture qui avait ensuite été réensemencée 3 fois dans un bouillon à glucose.

La souche 18 fermentait dans les deux cas la dulcité au cours de 72 heures.

Les souches 36, 76 et 80 fermentaient dans les deux cas la mannite au cours de 24 heures mais toujours sans formation d'air.

La souche 79 fermentait dans tous les deux cas la mannite au cours de 24 heures mais seulement avec un faible développement d'air.

Les souches 52 et 109 ne fermentaient dans aucun des cas la salicine au cours de 14 jours.

Dans une nouvelle expérience on a ensemencé les souches 18, 36, 52, 76, 80 et 82 sur une plaque à lactose et les souches 18, 76 et 80 sur une plaque à saccharose en partant des cultures préparatoires suivantes: 1) directement de piqûre 2) d'une culture de bouillon primaire 3) de la 3^e culture de bouillon 4) de la 3^e de trois cultures dont la première était une culture de bouillon et les deux dernières des cultures de bouillon-glucose. Dans plusieurs de ces essais on a observé des divergences par rapport à l'expérience principale décrite ci-dessus; ainsi, dans un cas, défaut de fermentation, dans un autre cas une fermentation lente; dans un essai une seule sorte de colonies, dans l'autre essai deux sortes. Mais entre les 4 séries ensemencées environ simultanément après des traitements préparatoires différents, la différence était moins grande qu'entre l'essai principal et les nouvelles expériences, et elles n'avaient pas un caractère régulier. Le ravivage au moyen de culture réitérée dans un bouillon ordinaire ou un bouillon à glucose ne jouait donc pas un rôle important.

XIV. LES FERMENTATIONS TARDIVES DE RHAMNOSE CHEZ SALMONELLA PARATYPHI B ET SALMONELLA TYPHI MURIUM

a. *S. paratyphi B*.

Comme démontré par *Kristensen & Bojlén* (voyez aussi *Kristensen*), les souches de *Salmonella paratyphi B* peuvent être divisées en plusieurs groupes, à savoir celles qui fermentent le rhamnose si vite que dans un verre il se produit une coloration en jaune au cours de quelques heures (R_1 et R_2), celles qui ne prennent la coloration en jaune qu'après env. 24 heures (R_3), et celles qui dans un liquide nutritif sont complètement négatives après 24 heures pour donner

ensuite une coloration en jaune après 48 heures ou plus tard encore, éventuellement pas du tout (R_4).

Pour examiner s'il s'agissait ici d'une fermentation typique ou mutative, dix souches R_3 (de différentes régions du pays) furent disséminées sur des plaques à rhamnose. Dans quelques-uns des cas il y avait une faible coloration en jaune après 24 heures; après 2 jours toutes les souches présentaient une coloration en jaune modérée; on n'observait pas de formation de bourgeons dans les colonies, pas non plus avec la loupe. Pour établir une comparaison, on fit une dissémination de 2 souches R_2 ; toutes les deux manifestèrent une coloration en jaune intensive au cours de 24 heures.

En outre on a examiné 2 souches qui avaient antérieurement été classifiées comme R_4 . De celles-ci l'une donna, dans un liquide nutritif à rhamnose, une coloration en jaune partielle au cours de 24 heures et devint tout à fait jaune dans les 24 heures suivantes; elle s'est donc conduite cette fois comme une souche R_3 ; en conformité, elle se comportait aussi sur une plaque à rhamnose comme les autres souches R_3 . L'autre souche R_4 , par contre, poussa sur une plaque à rhamnose avec des colonies bleues dans lesquelles apparurent des bourgeons jaunes, c'est à dire une fermentation typiquement mutative. Pour le diagnostic du type R_4 il faut exiger à l'avenir que la souche en question mute à la dissémination sur une plaque à rhamnose.

b. *S. typhi murium*.

A une certaine époque on regardait comme un fait caractéristique pour *S. typhi murium* que non seulement il fermentait le rhamnose au cours du premier jour, mais il le faisait aussi plus intensément que la plupart des souches de *S. paratyphi B*, à savoir comme le type R_1 assez rare du bac. paratyphique B (*Bitter, Weigmann & Habs*). Mais pendant la dernière dizaine d'années, les souches qui se comportent ainsi (c. à d. comme le type R_1 de *S. paratyphi B*) ont été plus rares (au moins dans ce pays) que les souches de *S. typhi murium* qui ne fermentent pas le rhamnose au cours de 24 heures, mais éventuellement après quelques jours. Il s'agissait maintenant d'examiner 1) si cette fermentation tardive de rhamnose était de nature mutative 2) si, dans l'affirmatif, on peut le regarder comme une qualité commune chez les souches, qui ne fermentent pas le rhamnose au cours du premier jour, qu'on peut les faire muter envers le rhamnose, bien que — à un seul essai ordinaire dans l'éprouvette — il ne se soit pas produit une fermentation au cours de 2—3 semaines.

A cette fin on a d'abord choisi 22 parmi les souches de *S. typhi murium* qui, à des examens précédents, n'avaient pas fermenté le rhamnose au cours de 24 heures.

Les souches avaient été isolées au cours des années 1932—43. 9 d'entre elles provenaient probablement de la même épidémie; les autres étaient de différentes contrées du pays. A l'essai fait antérieurement dans une seule éprouvette on avait observé les faits suivants:

5 souches fermentaient le rhamnose après 3—5 jours; les autres ne donnaient pas de réaction dans les périodes d'observation suivantes: 5 souches 1 jour, 4 souches 14 jours, 2 souches 20 jours, 6 souches 30 jours. En ce qui concerne les autres qualités fermentatives des souches, 2 d'entre elles fermentaient rapidement le d-tartrate; celles-ci donnaient toutes une réaction positive dans le bouillon à glycérine-fuchsine de *Stern*; les autres 19 souches fermentaient tardivement le d-tartrate ou ne le fermentaient pas du tout. Sur ces 19 souches, 14 donnaient une réaction positive et 5 aucune réaction dans le milieu de *Stern*. Aucune des souches ne fermentait l'inosite au cours de 24 heures.

Alors on a fait tant une dissémination sur plaque à rhamnose qu'un ensemencement dans un liquide nutritif à rhamnose. A une de ces méthodes au moins, on a constaté chez toutes les souches une fermentation mutative.

Une des souches donna à la dissémination sur plaque à rhamnose plusieurs formes qui différaient partie par l'aspect des colonies, partie par leur réaction envers le rhamnose, l'inosite et le milieu de *Stern*. Ensuite on a produit une culture unicellulaire de la forme originaire. Celle-là était positive dans le milieu de *Stern* le premier jour et ne fermentait pas l'inosite dans un liquide nutritif en 2 jours; cela était le cas tant avant qu'après la mutation envers le rhamnose. A un nouvel examen après 11 mois, il en était de même. Ici on n'avait donc pas non plus réussi à retrouver les variations à l'observation d'une culture unicellulaire.

Chez une autre souche c'était frappant que quelques-unes des colonies sur plaque à rhamnose contenaient de nombreux bourgeons, tandis que d'autres étaient sans bourgeons. Dans la progéniture de culture unicellulaire on trouva à la dissémination sur plaque à rhamnose une colonie riche en bourgeons, tandis que les autres colonies étaient pauvres en bourgeons. La différence entre le nombre des bourgeons dans les différentes colonies était trop grande pour être due à l'hasard. Nous voyons donc ici un exemple d'une dissociation dans des variantes avec une tendance mutative différente, c'est-à-dire le même phénomène qui fut soumis à une estimation numérique en ce qui concerne la mutation envers le xylose de la bacille typhique (vol. 20, p. 530 ff). Ces variantes semblent pourtant être peu constantes.

Bien que l'examen des 22 souches portât à croire que la mutabilité envers le rhamnose était une qualité ordinaire chez les souches de *S. typhi murium* qui ne fermentaient pas le rhamnose le 1^{er} jour, c'était pourtant intéressant d'examiner, si cette règle pouvait être confirmée au moyen d'un examen de matériaux choisis de souches qui, à un examen précédent, n'avaient pas donné une fermentation de rhamnose malgré une observation de longue durée. Des matériaux des années 1927—33, on a choisi 13 souches qui, à l'examen originaire dans l'éprouvette, n'avaient pas donné une acidification

en rhamnose à l'observation pendant 14 jours au moins; il n'y avait pas de relation épidémiologique frappante entre les 13 souches. Au nouvel examen toutes les 13 souches donnèrent à la dissémination sur plaque à rhamnose des bourgeons jaunes après 4—13 jours.

Les souches de *S. typhi murium* peuvent donc, au point de vue de leur réaction envers le rhamnose, être divisées en deux types: un type qui fermente le rhamnose rapidement et intensément, et un qui fermente le rhamnose d'une manière mutative; nous n'avons pas trouvé des souches qui étaient complètement incapables d'attaquer le rhamnose.

XV. LA FERMENTATION TARDIVE DE D-TARTRATE CHEZ *S. TYPHI MURIUM*

La plupart des souches de *S. typhi murium* qui mutent envers le rhamnose fermentent aussi le d-tartrate tardivement ou ne le fermentent pas du tout. On a l'impression que la fermentation tardive de tartrate est aussi en général mutative, puisque les réactions se développent souvent assez vite par rapport à la période passée sans signe de fermentation. Pour suivre l'apparition de la fermentation dans un certain verre il faut s'en tenir au «changement de couleur», c'est-à-dire la décoloration du bleu de bromothymol ajouté au milieu nutritif conjointement avec une croissance augmentée; le contenu du verre devient donc en même temps d'une couleur bleue plus faible et plus fortement trouble. Comme une fermentation faible et lente ne doit nécessairement pas entraîner un changement de couleur distinct, on ajoute à l'achèvement de l'essai 0,5 cm³ d'une solution aqueuse d'acétate de plomb saturée par verre d'env. 3 cm³. De cette façon, il se produit un précipité abondant, si le tartrate n'est pas fermenté; la fermentation se manifeste par le fait que le précipité devient moins abondant.

En essayant d'analyser la fermentation tardive de tartrate plus finement on se heurte à la difficulté que la forme qui fermente le tartrate et celle qui ne le fermente pas ne se distinguent pas assez clairement l'une de l'autre sur une plaque avec addition de d-tartrate. Ni une plaque à bactopectone-gélose + tartrate + bleu de bromothymol ni un milieu synthétique à sel d'ammonium + tartrate + bleu de bromothymol (avec ou sans addition d'un peu de glucose) ne se sont montrés appropriés. L'addition d'acétate de plomb n'a pas non plus mené au but visé, puisque les concentrations d'acétate de plomb qui rendaient la croissance possible étaient trop petites pour donner une précipitation distincte. Alors nous avons procédé comme suit:

4 souches de *S. typhi murium* à fermentation tardive de tartrate (1573, 1705, 1719, 1720) furent ensemencées chacune dans 6 verres à tartrate et observées pendant 7 jours, si une fermentation claire ne s'était pas montrée avant ce terme.

La souche 1573 ne donna en 5 verres aucun changement de couleur au cours de 7 jours, mais à l'addition d'acétate de plomb il se produisit un précipité qui n'était que $\frac{3}{4}$ de celui d'un verre non ensemencé. Il y avait donc eu une vague fermentation de tartrate; on ne pouvait pas décider si celle-ci était primaire ou mutative. Dans le 6^e verre, au contraire, une réaction prononcée se manifesta le 4^e jour. Déjà le fait que les verres d'une série ensemencée simultanément peuvent réagir d'une façon si différente fait supposer qu'il est question d'une fermentation mutative. Du dernier verre on fit une dissémination sur une plaque à gélose; 20 colonies furent ensemencées, chacune dans un verre à tartrate. Un de ces verres était positif après 1 jour; à l'addition d'acétate de plomb après 2 jours, on ne constata qu'un précipité peu important. Aux autres verres on ajouta de l'acétate de plomb après 5 jours de culture. Dans deux de ces verres, le précipité était d'env. $\frac{9}{10}$ de celui dans un verre non ensemencé (dans l'un de ces verres on avait constaté une décoloration faible après 4 jours, dans l'autre il n'y avait pas eu de changement de couleur). Les autres 17 verres ne manifestèrent aucun changement de couleur, et l'addition d'acétate de plomb après 5 jours donna un précipité aussi grand que dans un verre non ensemencé. Cet essai montre que la souche 1573 pouvait se décomposer dans au moins 2 différentes formes dont l'une fermentait rapidement le d-tartrate, tandis que l'autre ne montrait pas une fermentation sensible au cours de 5 jours. On pourrait, il est vrai, faire l'objection que les résultats variés des essais de fermentation dans chacun des verres soient dus à différentes conditions alimentaires dans les verres isolés, par exemple par suite d'impuretés qui pourraient ou entraver ou bien stimuler les pouvoirs fermentatifs. Quelque peu probable que soit une telle explication, on a pourtant tenu compte de cette possibilité à l'essai ci-dessous avec la souche 1720. Cependant, nous mentionnerons d'abord les essais avec les souches 1705 et 1719. Ici la période d'observation durait 7 jours; pendant ce temps on ne constatait pas de changement de couleur. Néanmoins le précipité était après l'addition d'acétate de plomb moins grand que dans le verre non ensemencé puisque, pour la souche 1705, il variait de $\frac{1}{3}$ à $\frac{3}{4}$ de celui du verre de contrôle; en ce qui concerne la souche 1719, il variait de $\frac{1}{10}$ à $\frac{3}{5}$. Les variations assez grandes de la quantité du précipité dans les verres ensemencés simultanément portent plutôt à croire qu'un élément mutatif entre dans la fermentation, mais n'en disent rien de décisif.

Les verres ensemencés avec la souche 1720 donnèrent une réaction positive aux époques suivantes: un verre après 2 jours, un verre après 3 jours, les autres 4 verres après 4 jours. Ici la différence de la rapidité de fermentation n'était pas si grande qu'il était de toute évidence qu'il s'agissait d'une fermentation primaire ou d'une fermentation mutative; en conséquence, on entreprit une dissémination sur une plaque à gélose du verre qui était positif après 2 jours. On obtint un mélange de colonies lisses et rugueuses; à l'agglutination sur lame avec sérum spécifique et non spécifique de *S. typhi murium*, 5 sur 8 colonies lisses se montrèrent spécifiques, 3 non spécifiques; 12 colonies rugueuses étaient toutes non spécifiques. De chacune des 20 colonies on ensemença un verre à tartrate. Tous les verres ensemencés des colonies lisses et le verre d'une des colonies rugueuses étaient positifs le 1^{er} jour; les verres des autres 11 colonies rugueuses donnèrent un faible changement de couleur le 5^e jour; à l'addition d'acétate de plomb en ce moment, on constata dans tous les verres un précipité moins grand que celui d'un verre non ensemencé, le premier variant de $\frac{1}{20}$ à $\frac{3}{4}$ du dernier. Ensuite on ensemença de 4 des colonies, chacune dans 10 nouveaux verres à tartrate, à savoir une colonie lisse spécifique, une colonie lisse non spécifique, la colonie rugueuse à fermentation rapide et une des autres colonies rugueuses. Les trois premières colonies donnèrent le 1^{er} jour une fermentation dans tous les verres. La 4^e colonie donna dans 3 des 10 verres une fermentation

(à en juger par la couleur) au cours d'un jour. A ces verres on ajouta, après 3 jours d'incubation, de l'acétate de plomb: seulement un précipité peu important. Les autres 7 verres ne montraient qu'un changement de couleur incertain après 5 jours d'observation; à l'addition d'acétate de plomb en ce moment, on constata dans tous les verres un précipité qui était de $\frac{7}{10}$ de celui dans un verre non ensemencé.

Le fait que la progéniture des 3 colonies »positives« donna une réaction positive dans tous les verres porte à croire qu'il s'agit d'une qualité chez les colonies et non pas chez les verres, tandis que les trois cultures positives parmi les 10 de la colonie »négative« peuvent s'expliquer par une mutation rapide; des examens ultérieurs étaient pourtant désirables pour obtenir une clarté complète sur ce point. Pour constater avec certitude que les variations se présentent aussi dans une culture pure sûre, on a produit une culture unicellulaire de la souche 1573 et 5 cultures unicellulaires de la souche 1720. De culture unicellulaire de chacune des deux souches on ensemença 20 verres à tartrate qui furent numérotés 1—20. Aucun des 20 verres avec No. 1573 ne montra un changement de couleur après 4 jours. A 10 de ces verres on ajouta alors de l'acétate de plomb. De ce fait se produisit partout un précipité aussi grand que celui du verre de contrôle. Les autres 10 verres furent observés pendant 14 jours au total, toujours sans changement de couleur. A l'addition d'acétate de plomb on constata un précipité de 0,7—0,75 de celui dans le verre de contrôle. Une fermentation faible avait donc eu lieu; on ne pouvait pas décider, si elle était de nature mutative. La culture unicellulaire de la souche 1720 donna le résultat suivant: Le 2^e jour les verres No. 1, 3, 6, 8, 12 et 14 donnèrent une réaction positive selon la couleur. On ajouta alors de l'acétate de plomb aux verres No. 1—10. Les verres No. 1, 3, 6 et 8 ne donnèrent qu'un précipité peu important, les autres verres un précipité aussi grand que celui dans le verre de contrôle. Au cours du 3^e—4^e jour, les verres No. 11 et 13—20 devinrent aussi positifs d'aspect; après au total 4 jours de culture on ajouta de l'acétate de plomb: partout seulement un précipité peu important. La culture unicellulaire de 1720 se comportait donc dans l'essentiel comme la culture originale.

Plus tard tant la souche 1720 originale que chacune des 5 cultures unicellulaires furent ensemencées, chacune dans 4 verres à tartrate. On constata dans les 20 verres des fermentations à des époques variantes, mais dans tous les cas au cours de 4 jours. A titre d'exemple nous mentionnons que sur les 4 verres à la culture unicellulaire No. 4, les deux étaient positifs le 1^{er} jour, tandis que les deux autres donnèrent un faible changement de couleur le 3^e jour et un fort changement de couleur le 4^e jour. De chacun de ces 4 verres on fit, après 2 jours de culture, un ensemencement ultérieur dans 6 verres à tartrate avec pipette Pasteur, une goutte dans chaque verre. Les verres ensemencés des deux premières cultures montrèrent tous un fort changement de couleur le 1^{er} jour, tandis que les autres verres ne donnèrent pas de réaction le 1^{er} jour; une telle ne fut constatée qu'à la vérification le 2^e jour. Maintenant on peut regarder comme tout à fait exclu que la différence entre une fermentation rapide et une fermentation lente est due à des conditions différentes dans les divers verres à tartrate.

Le caractère mutatif de la fermentation de tartrate chez la souche 1720 fut ultérieurement confirmé à une série continuée d'essais que nous laissons de côté; nous mentionnons seulement qu'on a observé des différences assez grandes quant à la rapidité avec laquelle les diverses souches fermentaient la mannite, car le moment de l'apparition d'une coloration en jaune complète variait de moins de 20 heures à 45—72 heures, tandis que la formation d'air variait de 0

jusqu'à $\frac{3}{5}$ du volume du verre intérieur. A l'agglutination sur lame avec les sérums O et H, toutes ces cultures se comportaient comme *S. typhi murium*.

XVI. DIFFÉRENTES FERMENTATIONS TARDIVES DANS LE GROUPE SALMONELLA

Comme supplément des fermentations mutatives dans le groupe *Salmonella* que nous avons examinées dans les détails dans les 15 chapitres précédents, nous avons fait un examen des autres fermentations tardives les plus connues dans ce groupe. Dans l'examen nous n'avons tenu compte que des cas où une souche fermentait un sucre si tardivement ou si lentement qu'il était permis de supposer qu'il était question d'une fermentation mutative, supposition qui — comme nous allons voir — ne fut pas confirmée dans tous les cas. Nous rangeons la matière par catégorie de sucres; quelques-unes des souches peuvent être trouvées sous la rubrique de plusieurs sucres.

1. *Mannite*.

Sur 4 souches de *S. typhi* suis, deux ne donnaient pas de fermentation au cours de 24 jours; deux mutaient envers la mannite, dans un cas sans formation d'air, dans l'autre cas avec une faible formation d'air.

2. *Dulcite*.

2 souches de *S. typhi* suis fermentaient toutes les deux de façon primaire, bien qu'avec une rapidité assez différente pour les diverses colonies.

6 souches de *S. cholerae* suis fermentaient toutes la dulcite d'une façon mutative. 4 de ces souches appartenaient au sous-type Kunzendorf, puisqu'elles formaient du H^2S et n'étaient agglutinées que par un sérum non spécifique; les autres 2 souches appartenaient au sous-type Amérique, car elles ne formaient pas du H^2S et étaient diphasiques.

1 souche de *S. gallinarum* fermentait la dulcite typiquement au cours du 1^{er}—2^e jour.

1 souche de *S. sendai* se comportait de la manière suivante: A la dissémination sur une plaque à dulcite, il se produisit deux sortes de colonies: l'une forma une zone bordière large et plate; la ligne qui séparait celle-ci de la partie centrale fut peu à peu moins distincte. Ces colonies restaient bleues. L'autre sorte de colonies était au commencement rondes et sans zone bordière, mais après quelques jours apparut une partie bordière qui était assez nettement séparée de la partie centrale ronde; cette dernière devint après 4—5 jours clairement jaune; dans une nouvelle dissémination, les parties jaunes firent

penser à des bourgeons. La différence entre la partie bleue périphérique et la partie centrale ou les »bourgeons« était cependant purement dépendante des conditions extérieures; car à la dissémination de chacune des trois parties on obtint des colonies qui étaient capables de former de l'acide, cependant seulement quand elles étaient particulièrement libres. Nous voyons donc ici une influence de nature singulièrement différente sur les conditions de l'acidification: dans la colonie seule, l'acidification n'apparut que dans le centre, tandis qu'à la dissémination on ne vit une acidification que dans la périphérie.

A un nouvel ensemencement, il y eut de nouveau un mélange de colonies bleues et jaunes; à l'ensemencement ultérieur toutes les deux donnèrent une faible acidification, mais seulement dans les parties bordières les plus libres.

On n'a donc pas pu constater une fermentation mutative proprement dite; la fermentation de dulcité chez la souche sendai doit être désignée comme primaire, mais sa rapidité peut varier dans une certaine mesure chez les différentes colonies.

1 souche de *S. moscou* et 1 souche de *S. enteritidis* var. chaco présentaient une fermentation primaire mais lente.

1 souche de *S. blegdam* donna, à la dissémination, un mélange de colonies à fermentation lente et de colonies qui ne donnaient pas de fermentation sûre; dans un liquide nutritif on constata en outre une forme à fermentation rapide.

1 souche de *S. enteritidis* var. essen fermentait d'une façon mutative.

3. Sorbite.

Sur 4 souches de *S. typhi* suis, 1 ne donna pas de fermentation, 3 souches fermentaient d'une façon mutative, l'une à développement d'air manquant ou peu important, la seconde avec jusqu'à $\frac{1}{7}$ de volume d'air, la troisième avec jusqu'à $\frac{2}{3}$ de volume d'air.

Une de ces souches était identique à une de celles qui montraient une fermentation primaire de dulcité, mais avec une rapidité variée. La mutation envers la sorbite était accompagnée d'une augmentation du pouvoir fermentatif envers la dulcité. Cela était démontré par la dissémination d'un matras à sorbite, dans lequel la mutation s'était produite, partie sur une plaque à sorbite, partie sur une plaque à dulcité. Sur la plaque à sorbite apparut un mélange de colonies bleues et jaunes (c'est-à-dire la forme originaire et la forme mutée); à l'ensemencement ultérieur sur une plaque à dulcité, les colonies jaunes fermentaient plus vite que les colonies bleues. Sur la plaque à dulcité on constata des colonies à acidification faible et forte; au réensemencement sur plaque à sorbite, ces colonies croissaient respectivement bleues et jaunes.

4. Xylose.

Trois souches de *S. gallinarum* var. duisburg mutaient envers le

xylose. Il est possible que la forme fermentaire pût en partie être trouvée avant l'entrée en contact de la culture avec le xylose.

En ce qui concerne la souche sendai, ce n'était pas facile de se faire une idée précise des conditions. De nombreux essais, soit avec la culture originaire, soit avec des cultures unicellulaires peuvent être résumés comme suit: On a observé la formation de bourgeons, mais à l'ensemencement ultérieur de bourgeons et de parties sans bourgeons on n'a pas pu obtenir deux formes constamment différentes. Lorsque les colonies sur la plaque à xylose avaient atteint l'âge de quelques jours, il apparut dans le centre de celles-ci une partie jaune en forme d'étoile dont la forme pouvait d'ailleurs différer sensiblement; dans certains cas les rayons étaient pointus, dans d'autres cas ils étaient obtus et toute la forme de l'étoile était très irrégulière. A l'ensemencement ultérieur une forme essentiellement similaire reparut, soit qu'on ensemencât de l'étoile, soit de la partie périphérique des colonies. Il s'agissait donc de conditions analogues à celles de la fermentation de dulcité.

5. *Rhamnose.*

Une souche *gallinarum* et une souche sendai donnèrent toutes les deux une fermentation primaire lente.

6. *Maltose.*

De *S. typhi* suis on a examiné deux souches qui, à des examens précédents, n'avaient pas fermenté le maltose. Une d'entre elles ne fermentait pas non plus cette fois; l'autre souche donna, au cours d'env. 3 semaines, un peu de coloration en jaune dans quelques-uns des verres à maltose ensemencés, avec jusqu'à $\frac{1}{15}$ de volume d'air. A la dissémination sur une plaque à maltose, il n'y eut aucune acidification. Nous ne pouvions donc pas décider, si nous avions affaire à une souche à mutation faible ou une souche à fermentation faible primaire. Que l'acidification fût due à une décomposition spontanée du maltose en glucose est moins probable, car dans ce cas il était permis de s'attendre à ce que l'autre souche formerait aussi de l'acide.

La souche sendai consistait en une forme qui fermentait lentement le maltose et une forme que le fermentait rapidement.

Une souche de *S. gallinarum* var. *duisburg* mutait, soit — comme la souche sendai — déjà avant le contact avec le maltose, soit en culture sur milieu à maltose (formation de bourgeons sur plaque à maltose).

Résumé.

1. 13 souches de bâtonnets ne prenant pas le Gram qui — lors d'un examen fait env. 12 années auparavant avaient fermenté la mannite avec développement d'air et donné une fermentation tardive tant

de lactose que de saccharose — ont été examinées, cette fois d'une façon plus approfondie, au point de vue de leur réaction envers le lactose et le saccharose. Chez une des souches on n'observa, cette fois-ci, aucune fermentation de lactose et chez 4 parmi les souches aucune fermentation de saccharose. Dans les autres cas on a observé ou une fermentation lente primaire ou une fermentation mutative ou bien toutes les deux; la fermentation mutative pouvait se développer sur la base d'une forme non fermentaire ou d'une forme à fermentation lente. Dans certains cas on a aussi observé une fermentation rapide chez quelques-unes des colonies dans la dissémination primaire. Ces différentes possibilités pouvaient être combinées de diverses façons, ce qui donnait, dans l'ensemble, une impression assez variée.

2. Les nouveaux essais quant à l'entraînement ont, comme les expériences précédentes, principalement donné un résultat négatif.

3. Les deux formes les plus lentes de fermentation de rhamnose chez *Salmonella paratyphi* B de celles décrites par *Kristensen & Bojlén* doivent être définies comme suit: R_3 = fermentation lente primaire; R_4 = fermentation mutative.

4. 35 souches de *Salmonella typhi* murium qui, à des examens précédents, n'avaient pas fermenté le rhamnose le 1^{er} jour et — pour 25 des souches — pas non plus en 14 jours, ont été examinées au point de vue de la fermentation mutative de rhamnose; celle-ci pouvait être provoquée dans tous les cas. Une fermentation lente primaire n'a pas été observée. A cause de sa réaction envers le rhamnose, *S. typhi* murium peut être divisé en deux groupes nettement distincts, à savoir les souches qui fermentent le rhamnose rapidement et intensément et celles qui mutent envers le rhamnose.

5. La fermentation tardive de d-tartrate chez certaines souches de *S. typhi* murium peut être de nature mutative; on n'a pas pu décider s'il existe aussi une fermentation lente primaire.

6. Une série d'autres fermentations tardives dans le groupe des *Salmonella* ont été examinées en vue de la question de fermentation mutative ou de fermentation lente primaire.

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Correction.

Dans la communication précédente (vol. 21, p. 214) on a omis, après la 5^e ligne, le titre du chapitre:

VIII. Production de mutations combinées.

ON OSTEOGENESIS IMPERFECTA

WITH SPECIAL REFERENCE TO THE PATHOLOGICO-ANATOMICAL CONDITIONS

By *Agnete Heise.*

(Received for publication November 2nd, 1944.)

The name osteogenesis imperfecta is due to the Dutchman Vrolik, 1849, and is the term most commonly used for congenital fragility of the bones and the accompanying condition, fracture upon quite minimal trauma. The affection had previously been called »foetal rachitis« or osteomalacia.

A closely related disease, now more and more regarded as identical with imperfect osteogenesis, is *osteopsathyrosis idiopathica*. In 1833 Lobstein had called brittleness of bone in general osteopsathyrosis, from *osteon*, bone, and *psathyros*, easily fractured, brittle. Not until later (Gurlt 1882) was idiopathic osteopsathyrosis distinguished as the form of brittleness in which no other affection can be demonstrated as the cause. Since it was Lobstein who had given an anatomical description of this particular form, it is his name, *maladie de Lobstein*, that has been associated with idiopathic osteopsathyrosis, which in England and America is generally known by the name of *fragilitas ossium*.

Common to Vrolik's and Lobstein's disease is the tendency to fracture of the bones, but the degree of invalidism, and even the viability, varies so much that the identification of these two diseases was not originally thought of.

Histologically investigated cases occurred as far back as 1859, when J. Schmidt's case, a foetus 4—5 months old, was microscopically examined by Wagner. In 1899 came Stilling with a microscopical examination of a foetus in the 8th month, in 1895 Buday, in 1902 Harbitz. The first Danish histological investigation was published in 1919 by Eiken, who mentions that there were in all 25 published histological cases.

Histological studies of patients with idiopathic osteopsathyrosis are exceedingly rare, but that of *Looser* in 1906 is classical. The patient was a boy whose first fracture occurred when he was 18 months old and who, when he was 17, had the lower extremities amputated. Looser ascertained that conditions were the same as in congenital osteogenesis imperfecta, and he therefore proposed the name osteogenesis imperfecta tarda for idiopathic osteopsathyrosis. The term was adopted without further qualification in Ziegler's and Aschoff's textbooks, but it also met with some opposition. Among its opponents was *von Recklinghausen*, who put forward the following points as characteristic of congenital osteogenesis imperfecta: 1) repeated fractures, 2) intra-uterine fractures, 3) structural changes of a kind to render the occurrence of spontaneous fracture understandable.

It was early realised that idiopathic osteopsathyrosis was hereditary, but its combination with blue sclerotics was not observed until later (*Spurway* 1895, *Eddowes* 1900).

Gradually as the interest in the subject grew, the following distinctions were drawn:

<i>congenital osteogenesis imperfecta</i>	<i>idiopathic osteopsathyrosis</i>
congenital	non-congenital
non-hereditary	hereditary
sclerotics natural	sclerotics blue

This, however, did not remain unopposed, and in the course of time the above delimitation has been found untenable. The predisposition was congenital, but the time of its manifestation was extremely capricious, and all kinds of transitions were observed, from intra-uterine or immediately post-natal occurrence to days, months, a year, or 18 months later.

Instances of hereditary brittleness of bone were described by *Ekman* in Sweden already in 1788. Ekman himself regarded the cases as osteomalacia, but doubtless we are here concerned with idiopathic osteopsathyrosis.

Since the publication of Eddowes' report numerous family trees have been published showing hereditary brittleness of bone and blue sclerotics. These cases are chiefly of a milder kind and do not cause any high degree of incapacity. For the severe intra-uterine cases, that is to say, congenital osteogenesis imperfecta, the general rule must be said to be that they occur sporadically, that the table of ascendants is negative. Almost unique is the case reported by *Smith* and *Mitchell* in which blue sclerotics were found as the only symptom in a family for several generations, after which a child was born who, in addition to blue sclerotics, had 14 congenital fractures.

Unique likewise is the case of *Puppel*, *Barron*, and *Curtis*, a 17

year old boy with fractures from birth and blue sclerotics. There were no previous cases, but a brother had blue sclerotics.

Children with congenital osteogenesis imperfecta as a rule die as infants and only a minority attain more than two years of age. In such instances there can be no transmission of the disease, but on the other hand nothing can be said as to descendants. This was the objection that could be raised against the abovementioned scheme which on this point proved untenable. In 1932 *Crooks* and in 1933 *Bierring* could report cases in which a woman with congenital osteogenesis imperfecta was delivered of a child by caesarian section, and in both instances a child with imperfect osteogenesis, which must thus be said to be hereditary.

With respect to the combination of blue sclerotics with congenital osteogenesis imperfecta, this condition has only been subjected to investigation in the present century, and more thoroughly only within the last 25 years. It then turned out that in numerous cases blue sclerotics were found combined with congenital osteogenesis imperfecta but that on the other hand this affection may occur with natural sclerotics, which must be said to be a considerable deviation from conditions in idiopathic osteopsathyrosis, in which the fractures are practically always accompanied by blue sclerotics, while in other members of the family these occur isolated.

The difference, however, cannot be considered so significant that a distinction between the diseases can be based on it; though of course it is not excluded that conditions so far unnoticed will later make such a distinction necessary.

Since 1912 attention has been directed towards a possible connection between fragility of bone and deafness. In that year, 1912, *Adair Dighton* published a genealogy comprising 14 persons through 4 generations. 9 individuals had blue sclerotics, and among these was a woman who, when 22 years old, became deaf after parturition. A longer record appeared in 1917 when the Scotch physician *Edith Bronson* reviewed the *Currie* family for four generations and demonstrated a hereditary predisposition to fractures and dislocations; a special form of skull with prominent frontal and occipital bones; blue sclerotics, and deafness which was diagnosed as *otosclerosis*. Since then several publications of a similar kind have appeared, thus in 1918 a report of two families with the triad: fractures, blue sclerotics, and deafness, by *van der Hoeve* and *de Kleyn*. Since, however, otosclerosis in the great majority of cases occurs as an isolated phenomenon, is no rare disease, and is itself a dominant hereditary factor, it will be difficult entirely to disregard the possibility that it may be an accidental combination.

Looseness of joints and a tendency to dislocation have often been described.

All these data made *Bauer* in 1920 maintain that the disease was

due to an inferiority of *all* the mesenchymal tissue, and this is further confirmed by his investigations of the *teeth*, which showed that it was the dentine which was affected. *Biebl* found similar conditions, but he also observed that the dentine first deposited was the most normal. *Birger Bergersen* likewise saw great changes in the dentine, but also in the enamel. At present the condition of the teeth in this disease is being subjected to a systematic investigation by *P. O. Pedersen* and *Jorgen Pindborg*.

A review of the literature shows that according to *Bamberg* and *Huldschinsky* the disease was first described by *Amand* in 1716 as »congenital brittleness of bone with a tendency to fracture.«

After these we have, according to *Frangenheim*: *Bordenave* 1763, *Henckel* 1772, *Sandifort* 1793. *Frangenheim* mentions only 10 publications in all up to 1889. During the last 50 years the number of published cases has steadily increased. In *Johannessen* and *Eiken's* work of 1919 the number of clinically observed cases of imperfect osteogenesis was estimated at about 150 in all, in *Max Schmidt's* work of 1925 the number of cases of osteopsathyrosis was estimated at about 220. *S. Kramer* (Würzburg) in 1939 records 600 literary sources — of congenital osteogenesis imperfecta as well as idiopathic osteopsathyrosis — in which some 1500—1600 persons are described. Since we are here concerned with the whole of the accessible world literature, he concludes that the disease is very rare. If, however, the publications keep on increasing in number at the same rate as hitherto, it will be necessary to change our view of the *extreme* rarity of the disease.

The case observed by *Amand* in 1716 was one of a foetus 4—5 months old suffering from »intra-uterine fractures without antecedent trauma«, thus a typical example of *congenital* osteogenesis imperfecta. The intra-uterine manifestation will always mean a much graver prognosis. Often the children are premature and the skull is merely like a membranous sack. — The defective development of the skull would seem to be directly proportional to the debility of the child. — Death in the child's first year of life is common, the cause of death being as a rule bronchopneumonia. As already mentioned, only a small number of the published cases refer to children more than 2 years old.

Among this small number may be mentioned: *Wernstedt* (1929), a girl of 10 with fractures from birth; *Simmons* (1907), a 13 year old girl; *Puppel, Barron, Curtis* (1935), a boy of 17. A few attain adolescence. *James Crooks* (1932), as previously stated, reports a mother with imperfect osteogenesis who was delivered of a daughter with the same disease by caesarian section, *Knud Bierring* (1933) a case analogous to that of *Crooks*.

The number of fractures varies greatly, from a few (rarely) to about 150. As a rule there is an abundance of callus which entirely

disappears again, and the fractures leave no traces on the spot beyond a tendency to curvatures which, on the other hand, may gradually become excessive.

Since the literature available has been published at intervals of years and in different countries, a survey is not quite easy.

It appears that there is full agreement with respect to the *very thin corticalis* which is without lamellae and the sparse trabeculae of the spongiosa, the architecture of which is entirely without system. As a rule it is stated that the bone can easily be cut through without preceding decalcification.

Of the description of the *provisional calcification zone* it is difficult to form an opinion, as some — the greater number — state that it is normal, others that it is absent. A comparison of the röntgenograms with the histological descriptions shows that this difference of opinion arises from the fact that by the provisional calcification zone some authors mean the narrow zone formed by the belt of cartilage cells — 3 or 4 cells high — marked in the röntgenograms as a fine shadow line, the epiphyseal line, while others include the region where there is still calcified cartilage in the trabeculae of the spongiosa, that is to say the so-called »cartilage-directed« trabeculae. It is the condition of these which differs very much from the norm, as they *very quickly perish*. This is mentioned among others by *Buday*, *Looser*, *Eiken*, and of course is particularly clear where a special cartilage stain has been used (*Hildebrandt* in 1889 used methylene blue combined with van Gieson's stain, while *Heise* in 1938 used methylene blue according to Fr. C. C. Hansen).

A single author, *Willon*, describes an *increase* of the ossification zone which can be observed macroscopically, and under the microscope proves to be derived from calcified, vesicular, degenerated cartilage. In this way a broad compact band is formed, while actual lines of direction are absent. This, however, is no doubt a temporary phenomenon and in a later stage may become »Wimberg rings«, which are mentioned in the same case; possibly arrest of growth from another cause *ad modum Harris*.

Very frequently the various authors have descriptions of cartilage islands scattered throughout the bones, particularly in the proximity of fractures, both periosteally and centrally. They occur with equal frequency in infractions and in fractures with displacement of the fragments. In *Lawford Knaggs'* 8th case cartilage islands in the skull are mentioned, and the author remarks that, »They would have been regarded as cartilage cells in any but a membrane bone.«

Observations of the transition of cartilage tissue to bone tissue by metaplasia are often referred to, and cells thus transformed are larger than usual and of a cartilaginous appearance, for which reason *Eiken* speaks of *chondroid* tissue, a term which he states he has adopted from *Ziegler's* textbook of pathological anatomy. The ex-

pression »chondroid tissue« must be said to be unfortunate, in so far as it is, in zoology, the name of a certain normal tissue. It is also used to describe young cartilage cells when callus is formed at the healing of fractures. In the present paper it will be employed about the bony trabeculae that, centrally, contain cartilage-like cells, of which, however, it is not known whether they are later changed into bone cells by metaplasia or simply die off. They correspond to Harbitz's description of »bony trabeculae which must be regarded as consisting centrally of cartilage around which the bone structure is disposed« (1902, a child born 4—5 weeks before term).

As regards the content of calcium it will be seen that, where staining with silver nitrate has been used for non-decalcified preparations, the blackened parts are of the same form and size as the bony trabeculae from decalcified preparations stained in the usual way. Some few authors have, however, spoken of the possibility of the presence of bone tissue without calcium. As to osteoid and halisteretic tissue opinions differ greatly. In some few cases indubitable marasmatic necrosis was seen.

Finally the condition of the *osteoblasts* has of course been very closely studied. They have, however, shown nothing characteristic of the disease either in number or form, having been present both in increased and in diminished numbers. But to those who have been used to regard the osteoblasts as the actual bone producers imperfect osteogenesis must necessarily be due to changes in the condition of these cells. They must therefore *show dysfunction*.

As to the genesis of the osteoblasts Økland has in Acta path. of 1940 put forward the following hypotheses: they originate

- 1) from histologically differentiated cells, fibrocytes or fibroblasts (there are also some authors who think that osteoblasts can be formed of much specialised bone cells),
- 2) from undifferentiated mesenchymal cells which are nearer to the embryonic tissue,
- 3) from lymphocytes or lymphoblasts.

Considering Leriche's vigorous contention that the role of the osteoblasts is secondary, a response to interstitial and humoral processes, it will be reasonable to refrain from expressing any opinion on these problems until physiological experiments bring about greater clarity, and like Clément, 1937, desist from ranging the osteoblasts in any causal connection as long as their place and role are not firmly established.

The condition of the marrow is extremely variable. In one bone fibrous marrow may be met with, in another lymphoid, in a third fatty marrow. The brittleness of the bones does not seem to be affected by the nature of the marrow.

The first *röntgen investigations* were restricted to the determination of the length and bowing of the bones. But already as early

as 1899 we have the following description of *Hildebrandt's* case (a child who died 8 hours after birth): bones short, clumsy, cylindrical, as thick in the middle as at the ends. Fractures partly fresh, partly old. The compacta only just indicated. The spongiosa irregular even where there is no fracture. The epiphyseal lines sharp, not always regular. The fractures as a rule show strong diastasis. Where there is no diastasis, no callus; at most a slight sclerotisation. The shadow intensity of the bones reduced. The callus shadow still fainter.

The other descriptions of conditions in infants agree with this in all essentials. The callus shadows, however, are as a rule rather marked.

If the children survive the following changes will be observed: the short clumsy bones grow more slender, the diaphysis even becomes narrower than at birth, while the epiphyses are broader and with a vesicular irregular structure which *Hirschmann* calls »grob-schaumig«. English authors speak of »foamy epiphyses«. According to *Fairbank's* observations the children retain the thick bones till at the latest the 8th month of life. In Denmark *Ruhwald* especially has called attention to the broad epiphyses and thin diaphyses, a condition which he, however, considered non-typical. The slender diaphyses and broad epiphyses have, in addition, been mentioned by *Arxhausen*, *Huldschinsky*, *de Cortes*, *Doering*, *Hektoen*, *Steinhauser*, *Stenvers* and *Stilling*.

The histological explanation of these röntgen findings is well expressed by *Looser*, who in his histological investigation of an amputated lower extremity of a 17 year old boy, whose first fracture occurred when he was 18 months old, has explained that the cartilage grows »independently of and regardless of the volume of the bone«. Where the case is as extreme as here, there are numerous breaks and disruptions of the doubled-down cartilage whose bone-producing side turns in all directions, even away from the diaphysis.

The fractures are oblique as well as transverse. They are as a rule followed by *bowing*. This bowing can be explained as a direct consequence of the fractures. In excessive degrees, however, yet another factor must be supposed to assert itself. As a rule this is regarded as osteomalacia, but in this connection it would be natural to think of the numerous cartilage islands.

Usually the hands and feet are spared. It is only as a rare exception that fractures in these parts are mentioned.

The as a rule *milder form in which the fractures did not appear till infancy* had, as previously stated, already been described in 1788 by *Ekman* (Sweden). His report dealt with a family with fracture of the bones in three generations. Not until a hundred years later did several publications appear in America and England.

As previously stated, a turning-point was reached with the Scotch physician *Eddowes'* report in 1900. He recorded a case of »Dark

Sclerotics and Fragilitas Ossium in a father and daughter. Though the dark, or as they later came to be called, blue sclerotics do not by themselves cause inconvenience of any kind, it was nevertheless the condition of the sclerotics which gave a further impetus to the study of the disease and stimulated the interest of the investigators of heredity. The blue sclerae occur when they are thinner than normal, and in 1913 and 1925 *Buchanan*, by histological investigations on blue sclerae, determined their thickness at $\frac{1}{3}$ of the normal in an adult and a $3\frac{1}{2}$ year old child respectively, both belonging to the Currie family (reported by Bronson). —

Through these thin sclerae the vessels are visible, and according



Fig. 1 a.



Fig. 1 b.

- a. The characteristically shaped head with bulging of the temporal regions,
 b. with an unduly prominent occiput.

After Puppel, Barron, Curtis.

to circumstances give them a bluish or slate-coloured hue. Close up to the cornea there is, however, a white ring. As the colour is not that of the sclerae themselves, the term blue sclerotics is in so far misleading, and *Max Schmidt* therefore has proposed the more correct term *leptoscleria*.

Eddowes' report was brief but exhaustive as regards the combination leptoscleria and brittleness of bone, and their heredity, and the name »Eddowes' disease« will therefore often be met with.

As to fractures and deformities there are as a rule some families with milder, and some with severer cases, but the cases may also be aggravated from generation to generation (*Kersley*), just as mild and severe cases may alternate without transitions. While the mild cases may be limited to some few fractures in infancy followed by quite insignificant deformities, there is in the severe cases a whole series of changes of which, again, some may be more dominant than others.

Table I.

	Case described by	Sex	Date of birth	Death	Relation to sisters and brothers	Heredity	Lep-to-sele-ria	First fracture
1909 1913 1925 1938	Fischer Monrad Max Schmidt Heise	M	4/11 26	24/11 06	No. 1	—	—	intra-uterine
1919	Johannessen Eiken	M	6/7 16	3/8 17 Broncho-pneumonia	No. 1	—	+	do.
1928	Heise	M	2/3 26	April 27 pneumonia	No. 2 of 2	—	+	do.
1931	Adolph Meyer	M	25/7 28	22/3 30 septicaemia		—	+	do.
1925 1930 1933	Max Schmidt Bierring	F	1/2 07			mother of next case	+	do.
1933	Bierring	F	29/10 29	April 30	No. 1	daughter of previous case	+	do.
1937	Ruhwald	F	28/4 25		No. 3 of 4	—	—	3 days old

In the severe cases the skull is stated to be a membranous sack. If ossification occurs up to 150 (*Vrolik*) and 172 (*Hektoen*) ossa Wormiana may be observed. Now and then exophthalmus is seen.

The shape of the skull is in many cases extremely typical, usually with a wide bitemporal diameter; but the fronto-occipital diameter, too, is not rarely prolonged and the occipital protuberances prominent (*Apert's* »crane à rebord»). The chin is pointed, and the ears are turned sideways above, downwards medially (Fig. 1).

The teeth may be gray and translucent, the cutting of the teeth irregular.

The spinal column will in most of the severe cases be deformed,

Table I.

Last fracture	No. of fractures	Deafness	Skull	Form of head	Teeth	Deformities of column	Deformities of extremities	Length	Weight	
not stopped	c. 30					—	+ +	40.5		
do.	c. 10		membranous, later bony			—	+		2350 g 6330 g	new-born 13 mths.
do.	numerous			characteristic			+ +			
do.			chiefly membranous, later bony				+ +	11 mths. 64 cm 14 mths. 67 cm	6130 g 7680 g	
18 years	numerous			characteristic			left arm right lower extremity	22 years 100 cm		
not stopped	do.		do.							
do.	do.					vert planac	+ +			

kyphotically as well as scoliotically. The corpora are described as very low, sometimes flat, sometimes hourglass-shaped.

Both as a result of the deformity of the column and because of the multiple fractures *the ribs* may show numerous deformities.

In the severe cases the pelvis is as a rule much deformed.

The extremities may have a complete corkscrew shape in the congenital cases. The femora are gradually bowed much outwards, the crura downwards and forwards.

The hands and feet are slender and well-shaped.

In comparison with the literature of other countries on this subject, not a few cases have been reported from Denmark, though we must be said to have come into the running rather late. The first

Table II.

	Case described by	Sex	Heredity	Leptoscleria	First fracture	Last fracture	No. of fractures
1902	H. Poulsen	M		not mentioned	3 fract. before 2 years		7
	do.	»	son of No. 1	do.	2—3 months		
	do.	»	son of No. 1	do.	4 months		
1921	Scheel	F	mother	+			5
		»	child	+			2
1921	Blegvad Haxthausen	»	+	+	childhood	13 years	7
1928	Eggert Møller	»	+	+	7 years	13 years	8
1929	Helms	M	father	+			4
	do.	»	son	+			3
1930	Harpöth	»	father	+			8
	do.	»	son	+			3
1933	Svensgaard	»	—	+	c. 3 years	14 years	7
1939	Henning L. Jensen K. K. Ortmann	F	+	+	14 years	36 years	8

case is from Rønne, Bornholm, published by *H. Poulsen* 1902 in *Ugeskrift for Læger*, p. 707, »Cases of Hereditary Fragility of the Bone System«. The published cases are distributed as follows:

Congenital fractures: See Table I.

Fractures occurring in infancy: See Table II.

16 cases in the same family published by *Holger Nielsen* in 1942: See Table III.

On reviewing the tables it will be seen that of the cases with congenital fractures only one out of 6 attained an age exceeding c. 1 year. On the other hand, this one woman, now aged 36, has given

Table II.

Deaf-ness	Skull	Teeth	Deformi- ties of column	Deformities of extremities	Age at exami- nation	Length	Serum	
							calcium	phosphorus
						"small"		
+								
			kypho- scoliosis		23 years	142 cm		
			—		29 years		12 mgr. %	
				—	adult			
					5 years			
					adult	157 cm		
					18 years	156 cm		
	charac- teristic	position irregular		left lower extremity 1 gr.	15 years	150 cm	10.6 —	3.83 mgr. %
+			—	—	36 years		9.9 — 10.2 —	

birth to a child with the same disease. With the exception of this child no one in this group had previous cases in the family. The mother has a height of 100 cm. Like all the others in whom the fractures have begun intrauterinely, she has numerous fractures and deformities. The seventh case in Table 1 is Ruhwald's patient. Her original fractures were not observed until 3 days *after* birth, but her malformations are monstrous and she is not able to leave her bed. She is 19 years old now. 5 out of the 7 had leptoscleria, among these the mother and child.

In the second table with 13 cases in which the fractures began

Table III.

	Sex	Age	No. of fractures	Leptoscleria	Deafness	Characteristic form of skull	Deformity	Hypermotility	Length
I 1	M	75 †	30	+	+				
II 1	»	58	25	+	+				
III 1	»	56	(2—14 years 20 last fr. 51 years	+	+	+		+	153
IV 1	F	28	(1½—27 years 8	+	+		+ r. radius	+	149
III 4	»	65	5 (last fr. 50 years	+	+		+ kyphosis	+	150
IV 2	M	8 †	(from 1½ years) 16	+	—				
IV 3	»	35	—	—	+				
IV 4	»	24	(1½—23) 10—12	+	+			+	
V 1		2	÷	+				+	"small"
IV 5	»	22	(1½—20) 6	+	+		+ extension defect in both elbows + kyphosis		155
IV 6	F	35	(.. 33) 5	+	+	+			
III 6	»	53	(.. 40) 3	+	+	+		+	147
IV 7	M	21	(as a child) 5—6	+	—			+	
III 8	»	5 †	(1...) 20	+	—				
II 4	F	50 †	10	+	—		+ kyphosis		
III 9	M	30 †	10	+	+				"small"

after birth, none has more than 8 fractures. Deformities are only found in 2, who have kyphoscoliosis and slighter degrees of deformity of the left forearm respectively. As to the last mentioned case, Svendsgaard's patient, there was no record of previous cases in the family, which must be said to be unique. The rest follow the general rule. For the first case — H. Poulsen's case, which dates as far back as 1902 — the colour of the sclerae is not mentioned. All the rest show leptoscleria, 3 are deaf. A single patient has an increased figure for serum calcium.

It should be added that the cases of *Blegvad* and *Haxthausen*, *Eggert Møller* and *Henning L. Jensen* and *K. K. Ortmann* all belong to the same family, in which there are 10 members presenting symptoms of the disease.

Harpøth's 2 patients belong to a family in which there are altogether 23 persons with more or less pronounced cases of the same affection. They are all small and gracile (height 150—162 cm.).

An inspection of the third table with 16 cases from four different generations in the same family shows that 8 had more than 8 fractures and of these 4 had from 20 to 30 fractures. 4 had slight deformities, none of these had over 10 fractures. All of them were short people. 12 were deaf, and of these one had this symptom isolated.

Further *Lindenov* in 1939 published a genealogy of a family in four generations. The dominant symptom is the blue sclerae, which were found in 25 members. Of these 7 had in addition hypermotility of the joints. Only one of them had as a child had 4 fractures after slight falls. This person is described as tall and strongly built. A few were hard of hearing, but the cause was uncharacteristic.¹⁾

Max Schmidt who in 1925 published »Some Cases of Blue Sclerotics and Brittleness of Bone« in the Medical Society of Jutland has been so kind as to offer me his material which in the main consists of patients with idiopathic osteopsathyrosis (or osteogenesis imperfecta tarda), but also includes some few cases of congenital osteogenesis imperfecta. Among these there was a child that died 20 days old at Dronning Louises Hospital for Children. The preparation from this child was kindly lent me by Professor *Monrad* for my investigations which, by the courtesy of Prosector *Vimtrup*, have been carried out at Bispebjerg Hospital.¹⁾

It was an *unbaptised boy*, who was born 14 days before term — 4/11 1906 — and died 24/11 1906 of pneumonia. No familial predisposition to the disease. Sclerae of normal colour. The *theca cranii* was membranous and only the regions round the 4 tubera and the protuberantia occ. ext. were hard. The fontanelles cannot be distinguished. *The upper and lower ex-*

¹⁾ After the submission of this, yet another case has been published, viz. Knud Seedorff, Osteogenesis imperfecta. U. f. L. 1944.

¹⁾ A preliminary report will be found in Hosp. Tid. 1938.

limbs present numerous deformities as shown by the following röntgen examination:

The skull flat posteriorly and with a wide bitemporal diameter. The shadows of the bones are so faint that the general impression is that of a membranous sack with some few bony islands. The lower jaw is small and slight, the teeth give only faint shadows, especially 3, 4, 5 which are merely «outlined».

The column: the corpora vert. lumb. comparatively low, otherwise normal conditions, apart from the atrophy of the bones.

Ribs: All the ribs show signs of fracture a little in front of the angle, some also behind the angle. On a level with the fracture the bone is broad and there are ring-shaped sclerotic areas alternating with porotic ones; in some few places fresh callus.

Upper extremities: The epiphyses somewhat broad compared with the diaphyses. On both sides numerous fractures which seem to be quite fresh. A little above the right wrist is seen a fracture of the radius with structural changes as in the case of the ribs.

The pelvis is somewhat rostrate in shape.

Lower extremities: The epiphyses broad compared with the diaphyses. There is fracture of both the femora, on the right a little below, on the left a little above the middle; on both sides abundant callus formation. The crura are strongly bowed with the convexity directed forwards. On the right a fracture a little above the middle with copious callus formation, on the left a little above the ankle.

The bones of the hands and feet are without fractures.

All the bones give very faint shadows, the corticalis showing merely as a faint outline.

Histological investigation.

Inferior part of left crus (Fig. 2).



Fig. 2.

Unbaptised boy. Pars inf. cruris sin. Magnif. $\times 3-4$. van Gieson.

The resting cartilage has fusiform cells corresponding to the age.

The height of the cartilage trabeculae about 20 cells.

The provisional calcification zone about 4 or 5 cells high.

It is normally calcified but the intercellular substance is very thin. Cartilaginous remnants are only found up to 1 mm. from the epiphyseal line. They are very sparse and very irregularly deposited. Transverse lines cannot be said to predominate. In some few places longitudinal deposition is preserved.

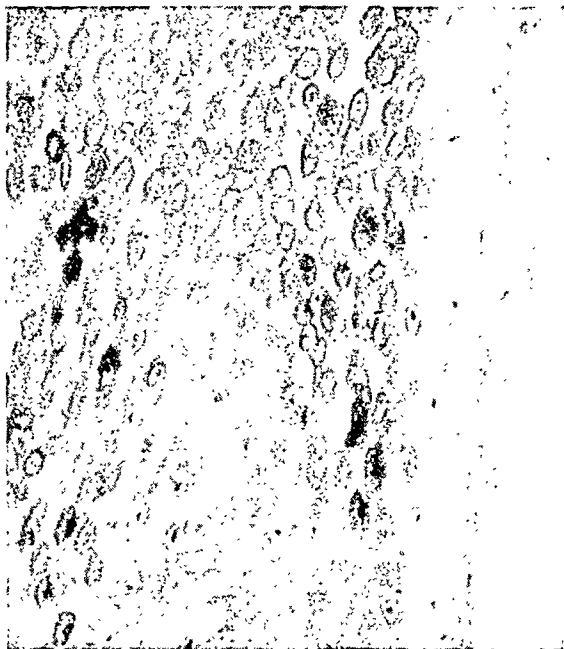


Fig. 3.

Unbaptised boy. Tibia dextr., diaphysis. In the middle the large chondroid cells, very numerous and closely placed, with the nuclei faintly and the capsule very conspicuously stained.

Surrounding this central block are seen the slender bone cells, placed at normal distance and with the nuclei conspicuously and the capsules faintly stained.

Methylene blue 1—5000.

The osteoblasts seem to be normal. The marrow immediately adjacent to the epiphysis is abundantly vascularised, and the lumen of the vessels is large.

The periosteal ossification is much influenced by an old fissure which can still be distinguished over 1/3 of the width of the bone 1 mm. from the epiphyseal line on the tibial side. On a level with it, by the tibial contour, a large area of cartilage is seen. By connective tissue septa this cartilaginous area is divided into 4 sections. One contains quite characterless cells, the other three cartilage cells placed longitudinally. In the peripheral areas there are signs of calcification. There is not, however, any enchondral calcification in the ordinary sense, as the cartilage is split up into areas containing accumulations of cells which have not perished. Around these the bone tissue proper is deposited in the usual way (Fig. 3).

Along the fissure degenerated cartilage and numerous osteoblasts are

observed. Between the cartilaginous area and the epiphysis on the tibial side are seen chondroid trabeculae from the periosteum. On the corresponding fibular side no bone formation at all is observed periosteally. In the metaphysis on this side sparse trabeculae occur now and then at right angles to the longitudinal axis. They have a chondroid appearance. At the transition to the diaphysis there is a marginal cartilaginous area. The diaphysis on this, the fibular, side shows a coarse reticulum of bone tissue. On the tibial side the bony trabeculae are placed transversely. Vessels are seen between them.

The periosteum is somewhat undulating. On a level with the cartilaginous area on the fibular side it is detached.



Fig. 4.

Unbaptised boy. Fracture in the middle of the right crus.
Hæmatoxylin — Eosin.

Fibula.

Cartilage and cartilaginous remnants as in the tibia.

1 mm from the epiphyseal line traces of an *old fissure* with a break in the contour and strands of degenerated cells running transversely.

From the *periosteum* chondroid trabeculae are developed as in the tibia, but here the cambium layer is also seen to become broader the further we get from the epiphysis, and this happens at the expense of the bone tissue. The broad cambium layer consists of degenerated tissue poor in cells and devoid of osteoclasts. 1 cm. from the epiphysis there is cartilaginous tissue from which issue chondroid trabeculae. In this place the bone as a whole is broken so it seems natural to regard the cartilage as post-fractural here. The chondroid trabeculae are chiefly coarse-meshed, but there also occur long straight trabeculae placed longitudinally.

Right crus.

Inferior part. Cartilage and cartilaginous remnants as in the other

preparations. Along the periosteum lies an extremely narrow strand of bone (1 cell broad). The marrow is very rich in cells.

Middle part. Fracture with angulation (Fig. 4). In the corticalis abundant formation of cartilage with cells placed lengthwise and peripheral calcification. From the cartilage issue chondroid trabeculae towards the periosteum as well as towards the marrow. On the convex side the chondroid trabeculae form a prominence periosteally. Between the trabeculae is seen connective tissue with very sparse cells. In some few places, however, there are traces of areas richer in cells.

Metatars. Cartilage and cartilaginous remnants as in the other preparations. The trabeculae chondroid, sparse with osteoblasts of normal appearance in the contour. There are numerous osteoblasts. Of this preparation sections were taken of non-decalcinated tissue, and it is seen that the calcium lies in the narrow margin of calcified cartilage and the chondroid trabeculae. In none of the preparations is there any trace of lamellar structure.

Summary.

Röntgen investigation.

Numerous costal fractures.

Column straight.

Pelvis somewhat rostrate.

The epiphyses of the long bones broad, the diaphyses slender.

Fractures of the upper and especially of the lower extremities where there is considerable deformity and abundant callus.

General atrophy of bone.

Histological investigation.

The provisional calcification zone is thin but normally calcified.

Cartilaginous remnants only found in a breadth of 1 mm.

Numerous islands of cartilage and chondroid trabeculae, which form a quite irregular structure and are outlined by bone cells. Nowhere a normal osseous architecture.

Case G. C. B. was given me by the Children's Department of the Rigshospital, for which I owe thanks to Professor *Bloch*.

The little girl died on the 24/1 1933 of mucopurulent bronchiolitis after having been two days in the department. The child was then 15 months old. No familial predisposition to the disease. No. 2 of two children. The brother in good health. She had not cut her first tooth till she was 14½ months old. The upper and lower extremities much deformed.

Section showed the following particulars: emaciated, small, slightly built.

Thorax much deformed with everted curvatures and very prominent sternum.

Skull very large, especially broad, as broad as long: anterior fontanelle large, measures about 5 × 6 cm., bones of skull quite thin and brittle.

Extremities much deformed, bowed and shortened, most in the upper part of the arms and crura; the bones very thin and brittle, »porous«.

Fatty tissue sparse.

The thymus weighs 23 gr.

Lungs hyperaemic, full of fluid, but everywhere containing air; a good deal of mucopurulent secretion extends into the finest branches of the bronchi.

Heart natural in form and size, no deformities, the oval foramen closed.

Liver, form, size, and consistency natural. Pea-sized cyst in the left ovary.

Theca cranii has very thin bones: the cerebrum large, nothing macroscopically abnormal, no hydrocephalus. (Husted).

The röntgenogram showed:

The upper extremities are bowed, with the convexity outward. The diaphyses are thin compared with the epiphyses. As far as the humerus is concerned, the central thin area only constitutes $1/5$ of the whole length of the bone. In the bones of the forearm the slender area is comparatively longer. In both humeri sclerotic strands indicate old fractures. In the middle



Fig. 5.

G. C. B. Left tibia, pars inf. Magnif. $\times 130$. Hæmatoxylin — Eosin.

of the left radius is seen a comparatively fresh fracture with copious callus formation.

The bones of the hand are of normal shape.

Practically all ribs show signs of fractures.

The column has low narrow corpora, no scoliosis.

The pelvis has slightly prominent acetabula, not rostrate.

The femora are somewhat twisted in the middle which is not conspicuously slender. The structure indicates old fractures. The crura are bowed with the convexity anteriorly.

All the bones give faint shadows, the corticalis, especially, only presents itself as a faint contour.

For the histological examination at the Bispebjerg Hospital I had the inferior part of the left tibia. It showed:

The cells of the resting cartilage are fusiform and placed close together. The cells of the cartilage columns are somewhat flattened. Their number is about 20. The calcification zone does not differ from the rest of the cartilage by any other colour. The septa of the columns are very thin and do not

persist long as cartilaginous remnants of which only a very few are seen, indeed so few that you have to search for them.

The bony trabeculae in the *metaphysis* are in complete confusion, only forming a border towards the calcification zone. They are narrow, and surrounded by a row of osteoblasts of normal appearance.

The *periosteal bony trabeculae* are narrow and sparse and show no traces of lamellar structure. The form of the bone cells is similar to that of genuine bone cells.

The marrow contains a connective tissue poor in cells which surrounds all the bony trabeculae and lies close up to the calcification zone (Fig. 5). Centrally, fatty marrow is seen, here and there with lymphoid elements.

Summary.

The röntgen examination:

The thorax deformed, numerous costal fractures.

The column has low narrow corpora.

The diaphyses of the upper extremities are thin compared with the epiphyses. Both the upper and the lower extremities show traces of old fractures and deformities.

General atrophy of the bones and complete lack of compacta.

Histological examination of the inferior part of the left tibia:

The calcification zone slender, otherwise normal.

Number of cartilaginous remnants minimal.

No cartilage islands or chondroid trabeculae.

The periosteal bony trabeculae are narrow and sparse, otherwise normal.

No lamellar structure.

All bony trabeculae are surrounded by a connective tissue poor in cells.

Centrally is seen fatty marrow, here and there with lymphoid elements.

Case N. J. was kindly given me by Professor *Oluf Andersen*.

This was a girl aged 2, born 6/8 1940. Twice admitted to Dronning Louises Hospital for Children — 16/9—21/9 1940 and 20/6 1941 till her death 11/7 1942. No familial predisposition to the disease. The first fracture — of both femora — occurred when she was 1 month old, the last — of the left femur — 2 months before death. Likewise fracture of the crura, but not of the upper extremities. In the last year of life the temperature varied, as a rule rising to 40°. Only some few periods without fever. The urine was occasionally positive for albumin and bacteria, otherwise nothing demonstrable as the cause of fever. Serum calcium slightly increased — 12.5—12.2 mgr. %. After ingestion of 100,000 D units daily + 16 drops of detrimine a further rise in the serum calcium to 19.2 mgr. %.

The parathyroid gland larger than normal but without adenoma. After parathyroidectomy in two tempi normal figures for serum calcium.

The effect on the fractures, which did not cease, cannot be estimated, as the patient died 2 months after the last operation.

Length on 12/4 41, 56 cm.

Length on 12/2 and 27/4 42, 63.5 cm.

The increase in length seems to have ceased in the last 6 months.

The skull broad with a considerable amount of ossa Wormiana.

Sclerae blue.

Teeth amber-coloured.

Trunk natural form.

Röntgen examination 1940. All bones give faint shadows. By the skull a considerable amount of ossa Wormiana are seen.

The upper extremities, ribs, and column present nothing special beyond the atrophy.

The femora show fractures at the transition between the upper and middle 1/3. There is outward curving and some callus formation.

The left crus shows fracture in the middle with outward curving:

The diaphyses are not conspicuously narrow. Nowhere any trace of bulging. No cysts can be demonstrated.

Section. 13/7 42. Adenomata parva gl. parathyreoid.?

Pronounced softness of bones.

Hyperaemia pulm. med. gr.



Fig. 6.

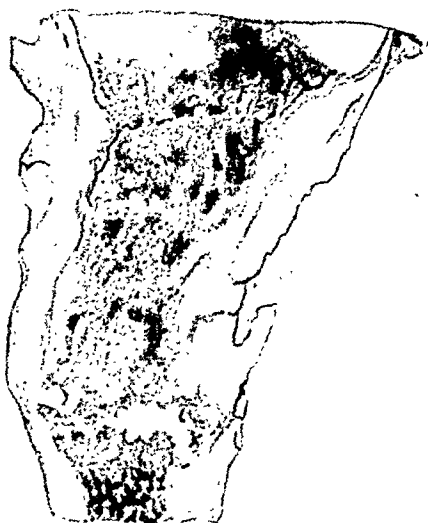


Fig. 7.

Fig. 6. N. J. X-ray of the left femur, upper part.

Fig. 7. N. J. Left femur, drawn by Bodil Strubberg after section corresponding with a part of fig. 6.

11/1 43. Microscopical data: No parathyreoid tissue was found. In the vicinity of the gland only moderate inflammatory changes. Microscopy of the ribs showed a picture corresponding to osteogenesis imperfecta.

The upper part of the femur was given me for a special röntgen examination. By the courtesy of Prosector *Petri* I carried out the histological examination at the Kommunehospital.

Röntgen examination.

Upper part of femur. The bony nucleus of the head is well developed, its contour distinctly marked, the structure somewhat irregular.

The epiphyseal line of the chief bone is regular and well delimited. It forms no contrast to the rest of the structure, but passes smoothly into the gracile metaphysis in which mottled clearings are seen. Just below the trochanter the intensity of the shadow is very faint over a zone of about $\frac{1}{2}$ cm. There is a break in the contour medially, but no sign of fresh fracture or reaction to older fractures. From here to a couple of cm. distally, however, the osseous strands show, as it were, angulation, and at the distal end of this area is seen a transverse fracture in process of healing with spur-like prominent callus at the edges.

Ribs. The epiphyseal line forms a very sharp contrast with the gracile bone. A couple of mm. from the epiphyseal line there is an infraction with a somewhat woolly structure in the vicinity. A preparation from the skull shows structure.

For *decalcification* was used 5% nitric acid. After the preparation had been immersed for 20 hours, the röntgen examination was repeated. It showed: The bony nucleus of the head decalcified, the metaphysis almost decalcified, the shadow-giving part of the rest of the bone limited to half its width, chiefly at the expense of the lateral parts; the *ribs* only show traces of calcium shadows in the area most distant from the cartilage; in the preparation of the skull only doubtful remnants. 41 hours' decalcification shows complete decalcification of the rib and skull preparations, in the femur are merely faint shadows some few places in the centre of the bone. After 65 hours the femur was completely decalcified.

Macroscopical: The preparation of the upper part of the femur was then divided into an upper, middle, and lower piece. These were again divided into frontal sections, the lower part of the upper piece also into horizontal sections. At the longitudinal division a regular transition from cartilage to bone is seen. The tissue below the trochanter region is very soft and shows traces of a transverse fracture which seems fresh and is without signs of periosteal reactions. A few centimetres further down is seen an old transverse fracture with much cartilage in the callus, which is prominent at the level of the fracture line. (Fig. 7). Longitudinal and transverse sections of the femur have been treated with paraffin, the longitudinal sections also with celloidin. Of the preparation from the ribs longitudinal and transverse sections were taken which were mounted in paraffin. Of the preparation from the skull, surface and transverse sections were mounted in paraffin. Besides the surface sections were mounted in celloidin.

Microscopical:

Ribs: Resting cartilage normal. The columns of the proliferating cartilage are about 20 cells high, the columns of the provisional calcification zone are about 6 cells high. Between the longitudinally placed columns of the calcified cartilaginous remnants is seen granulation tissue rich in cells. The calcified cartilaginous remnants persist to an extent corresponding to twice the height of the provisional calcification zone, but then cease abruptly and in this place there seems to be a fissure with bleeding. Along one contour of the bone is seen a narrow cambium layer, from which issue thin bony trabeculae, and between these and the marrow lies a zone of fine connective tissue with very open meshes. The width of this zone is the same throughout its extent. On the other side of the bone the trabeculae are still thinner and not continuous. They lie like small ornaments in a broad cambium layer. Here, too, there is a border of fine connective tissue towards the marrow, which is predominantly lymphoid and very rich in cells. In the area nearest to the above-mentioned fissure it is fibrous.

Upper end of the femur.

Histological findings.

Resting cartilage normal. The columns of the proliferating cartilage about 20 cells high, the provisional calcification zone about 5 cells high. In some places a quite short railing of longitudinal cartilage columns persists; they hardly exceed the provisional calcification zone in length, and in between them is seen granulation tissue. Most frequently, however, such a railing is lacking, and the marrow lies close up to the provisional calcification zone. In the metaphysis extremely sparse bony trabeculae are seen, running equally longitudinally and transversely. Only as a rare exception are remnants

of calcified cartilage observed in these trabeculae. More distally the corticalis of the metaphysis is extremely narrow. Further below on the lateral aspect of the diaphysis it becomes comparatively broad.

Corresponding to the place of the small trochanter is seen a break in the contour with indentation of the periosteum, and from this a line running transversely shows traces of exudation. On either side of it there are small osseous trabeculae which in some few places contain calcified car-



Fig. 8.

Left femur, upper part. Magnif. $\times 130$. Hæmatoxylin — Eosin.

tilage. Then comes an area in which the periosteum is undulating and with another break in the corticalis and transverse strands characterised by fibrous areas which suggest a transformation after a fissure.

2 cm. distally to the trochanter are seen traces of a transverse fracture in process of healing. Both periosteally and endostally cartiliginous areas are seen, partly in fibrous degeneration, partly in course of transformation to osseous tissue, and this takes place both enchondrally and as a cover of the chondroid trabeculae. At the level of the fracture-line, on both sides, there is a spurlike prominence on the periosteum in which is seen chiefly calcified cartilage, but also osseous trabeculae.

In its *lateral half* the marrow is rich in cells upwards, then more fibrous, containing cartilage cells at the place of the fracture, then it is again fibrous and finally also lipoid. In its *medial half* it is fibrous upwards, interspersed with lipoid areas, then very much marked by the fissures

of various dates which here extend into the bone. *Osteoblasts* cannot be demonstrated with certainty in the immediate vicinity of the provisional calcification zone. In the osseous areas of the metaphysis and further distally they seem to be of normal appearance.

A very remarkable appearance is presented by the bony trabeculae with large closely placed cells which on staining with methylene blue show a distinct colouring of the capsule as a sign that they were originally cartilage cells. (Fig. 8). In the marginal zone are seen cells of a more osseous appearance, and along the edge osteoblasts. There is no lamellar structure anywhere.

Summary.

Röntgen examination.

General atrophy of the bones and old fractures of the lower extremities. The femora deformed. The diaphyses not conspicuously narrow.

Numerous ossa Wormiana.

Histological investigation of the ribs.

Provisional calcification zone slight.

Calcified cartilaginous remnants only persist for a short time, interrupted by fresh fissures. No callus. Periosteal bony trabeculae slender, surrounded by a fine connective tissue.

Upper end of left femur:

Provisional calcification zone slight.

The cartilaginous remnants in some places persist for a very short time, in others they are entirely absent. There are traces of at least three fractures, among these — 2 cm distal to the trochanter — a transverse fracture in course of healing with abundant calcified cartilaginous callus, which is in process of transformation into osseous tissue, enchondrally and covering the chondroid trabeculae. The rest of the bony trabeculae show cartilage-like cells centrally. No lamellar structure anywhere. The marrow is rich in cells and fibrous, strongly marked by the numerous fractures.

We are thus concerned with three children which presented clinical symptoms of congenital osteogenesis imperfecta, and one of them likewise of parathyreoidism. Histologically the cases resemble each other in the *rapid destruction of the cartilaginous remnants*, the *sparse bony trabeculae*, and the *complete lack of a normal structure*. For the two with fresh fractures, the unbaptised boy and J. N., is seen *abundant formation of cartilage and chondroid trabeculae*. As far as the two eldest are concerned, G. C. B. and N. J., the *fine strands of connective tissue* disposed round the bony trabeculae are conspicuous.

For all the cases the result is extraordinarily gracile bones with a quite thin corticalis. In this respect they remind one of the conditions in scurvy, but in spite of severe fractures periossteal bleeding is nowhere seen, and a feature differing greatly from the conditions in scurvy is the abundant development of cartilage. (According to *John Hertz*, no cartilage is formed during the healing of the frac-

tures when there is vitamin C deficiency). Nor it is a case of simple atrophy, as shown by the structural changes, and there are no traces of *ostitis fibrosa*.

As a feature peculiar to this disease we must regard the rapid destruction of the cartilaginous remnants (Fig. 9). For comparison with conditions in *normal* subjects their persistence has been in-



Fig. 9.



Fig. 10.

Fig. 9. Unbaptised boy. Right tibia, pars inf. Magnif. $\times 9$.
Methylene blue 1—5000.

Fig. 10. Normal foetus, 8 months old. Tibia, pars inf. Magnif. \times ca. 4—5.
Methylene blue 1—5000.

vestigated in preparations from the lower part of the tibia (Figs. 10 and 11), in the case of the adult subject from the first metatarsus.

The results are as follows:

8 months old foetus: Throughout the metaphysis the calcified cartilage forms closely placed longitudinal columns covered with bone tissue (the diaphysis is not seen in the preparation).

Full-term foetus: Throughout the metaphysis the calcified cartilage forms closely placed longitudinal columns covered with bone tissue, the bony trabeculae of the diaphysis devoid of cartilaginous remnants.

8 year old boy: To a height of about 7 cells the calcified cartilage forms closely placed longitudinal columns, several of which are covered with bone tissue; in the trabeculae of the metaphysis there are only very scattered cartilaginous remnants.

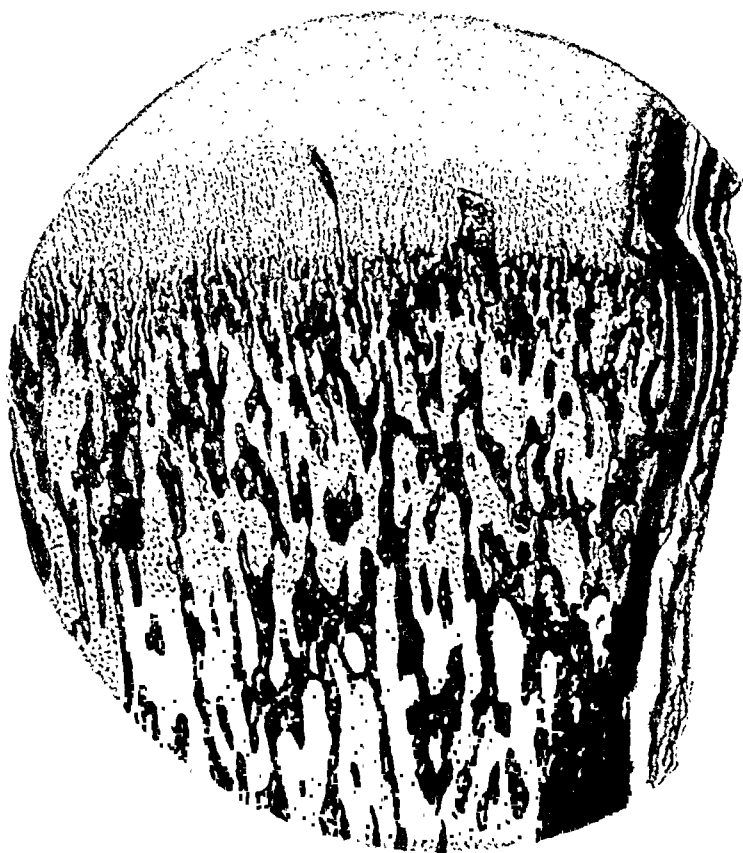


Fig. 11.

Normal 8 months old foetus.

Tibia, lower part. Painted by Bodil Strubberg. Magnif. $\times 22,5$.
Methylene blue 1---2500, molybdic ammonia, acid fuchsin + picric acid.

Adult: Continuous transverse bony trabeculae along the cartilage of the joint, no calcified cartilaginous remnants.¹⁾

The conditions described thus differ greatly from the normal development and have, indeed, been observed previously. In 1893 *Sigurd Moller* mentions that the cartilaginous remnants quickly perish. In 1895 *Buday* states that the cartilaginous remnants are only found to a width of 2 mm., and in 1906 *Looser* says that the cartilaginous remnants disappear more rapidly than normal. In 1913 *Frangenheim* arrives at the same result as *Buday* and declares that the cartilaginous remnants ought to have persisted to the middle third of the bone.

Thus the cartilage model formed of the calcified cartilaginous remnants perishes at the very start.

With respect to the second peculiarity, the *chondroid trabeculae*, it is not present in the case of G. C. B. That it may be due to a relative standstill — no fresh fractures — is possible and might be conceived to indicate a connection between the forming of chondroid trabeculae and the very abundant formation of cartilaginous callus in the fracture periods. About the development of cartilaginous callus there is, however, a great difference of opinion; some authors think that cartilaginous callus in itself is something irregular, while others, thus *Lauche*, *Schaffer*, *Kapsammer*, *Roux*, *Giani M. B. Schmidt*, *Pollicard*, are of opinion that it occurs when there is dislocation of the fragments, not otherwise. And it is in fact always found on a level with the line of fracture, not like the osteoid, beginning at some distance from it. In osteogenesis imperfecta it also occurs where there is no dislocation, and in striking abundance. This may perhaps explain the tendency to fractures in the same place, since the same resistance cannot, probably, be ascribed to calcified cartilage as to bone tissue. Nor is it probably excluded that these conditions may be connected with the bowing following the fractures.

As to the lack of growth in thickness, of the lamellae, we can only note its absence.

And finally as regards the cell-poor marrow it is difficult to estimate whether it is a primary or a secondary factor. In some places it may have the appearance of primitive connective tissue, which does what it can, but is little productive; in other places it conveys the impression of final stages. *Harbitz* says about the marrow that it is most like lymphoid marrow without, however, being so rich in cells. He further describes a marrow rather poor in cells of

¹⁾ The preparations of the tibia in the embryos are derived from the section room of the Rigshospital and have been given me by the courtesy of Professor *Poul Moller*. The preparation of the tibia in the 8 year old boy *Mar Schmidt* has kindly given me. It is derived from an amputation in *Horsens Kommunehospital*. The preparation from the adult subject, too, is from an amputation and has been given me by the courtesy of Professor *Chievitz*.

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The reader is further referred to the list of literature in Kramer's work.

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